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THE PAST BETOKENS THE FUTURE

A KNOWLEDGE of past events is of the utmost value when one essays to prognosticate upon the future and its probable requirements. It has been said that "History repeats itself." There can be few people who could not recall events and occasions in their own lives or even in the wider spheres of national and international affairs which would lend substance to this aphorism. Henry Ford the first has been quoted as saying that "History is bunk," but his success in life and the inestimable benefits which have followed his development of the automobile are striking examples of the value of history. Ford did not invent the internal combustion engine, neither did he invent the wheel nor the pneumatic tyre. Without these epoch-making basic discoveries there could have been no motor cars. Ford, like other men, profited from the lessons of the past and the genius of their work was to develop and modify something which was already known. The telephone is now ubiquitous throughout the world, yet this wonder originated in the relatively simple machine which was conceived by the brain of Dr. Bell, who is reported to have said that although he made the first telephone he could not explain how or why it worked. These examples demonstrating the value of a knowledge of history as an essential adjuvant to progress have been taken from the industrial and mechanised world, but in the realm of scientific endeavour which embraces the disciplines of human and veterinary medicine there are many similar illustrations of equally vital importance.

(The December issue of The British Veterinary Journal was published on December 2)

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The art and craft of medicine and surgery has soared to fantastic stature in recent years and the impetus to advance shows no signs of stay. This is due in the main to the elucidation of the nature and manner of many of the metabolic activities within the animal body which have been disclosed by the patient efforts of the Anatomists, Physiologists and Chemists. Would this advance have been so great if there had not been a Harvey or a Claude Bernard? Pathologists and Bacteriologists have been able to explain many of the mysteries of the various disease processes which can lay the body low. In this instance we owe a great debt to such men as Pasteur, Turnbull, McFadyean and W. Bulloch, who delved so diligently into the unknown and recorded their observations.

Mankind will be forever in the debt of the men who took the initial steps to promote a state of general anæsthesia, and this in the face of professional and religious opposition. All modern anæsthetic methods stem from the premier work of Hickman, Wells, Morton, Simpson and Squire and of Warren and Liston, the surgeons who were courageous enough to give the new idea a practical trial. Anæsthesia abolished the immediate terror of the operating theatre but, alas, post-operative sepsis was uncontrolled and took a terrible toll. Lister and his disciples, profiting by the work of Pasteur, and again in the face of professional opposition, pioneered the road which has led to the brilliant surgical expositions of to-day.

All regions of the body now come within the province of the surgeon, and the patient has been made safe for the surgeon and the surgeon made safe for the patient.

We have mentioned but a few of the great men who were bold and inspired explorers in the realms of the unknown. There are many others who also pursued an ideal in the search for truth and thereby laid the sure foundation on which progress could be made. The story of their lives and work, their triumphs and disappointments, their persistence in spite of the rebuffs and censure of their contemporaries, makes entrancing reading and acts as a "spur to emulation" in their successors.

Veterinary Science is not lacking in its great men of the past whose work has brought renown to themselves and to the profession. Sir Frederick Smith has told their story up to the beginning of the present century. He undertook and accomplished a gigantic task and thereby left an indelible mark in our annals. His was a labour of a love and respect for the past and an expression of his supreme conception of his duty to those who may follow. He worked to within a few hours of his death. The time is ripe for other hands to grasp his torch and carry on from that sad day when, he being old and frail, the great Reaper called this faithful servant of the profession to his rest.

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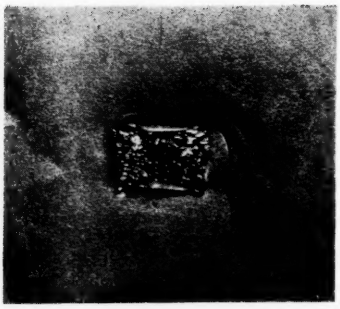

Fig. 7(*a*)
Portion of small intestine showing typical lesions (natural size).

Fig. 7(*b*)
Section of diseased mucosa showing necrotic villus. H. & E. x 64.

Fig. 7(*c*)
Higher-power magnification of junction between necrotic and normal mucosa as shown in Fig. 7(*b*). H. & E. x 200.

(*Article by Ford, page 3*)

GENERAL ARTICLES

A SURVEY OF TURKEY MORTALITY

By E. J. H. FORD, B.V.Sc., M.R.C.V.S.*

Boots Pure Drug Co., Ltd., Veterinary Science Division, Thurgarton, Notts.

Introduction

DURING the course of investigations into suspected outbreaks of histomoniasis in turkey flocks, it became increasingly evident that many flock owners were under the impression that *Histomonas meleagridis* was the main if not the sole cause of disease in turkeys.

The results of these investigations suggested that many of the outbreaks were due to causes other than blackhead and it was also evident that whilst a variety of turkey diseases had been described in the literature there was little information on the relative incidence of various conditions in Great Britain. In the U.S.A. Dearstyne (1945) gave the mortality records of one flock and Bullis (1945) has analysed the relative incidence of various diseases encountered during the examination of 483 consignments of carcases received over a ten-year period. Some of their findings together with those of Gordon (1955) in this country will be discussed later in the paper.

Methods

In order to obtain information on relative mortality in this country a number of turkey flock owners were asked to co-operate by submitting all dead birds for post-mortem examination and also providing detailed stock returns. It was felt that the examination of all carcases from a number of farms was the only way to obtain a real picture of the relative importance of various diseases as the examination of diagnostic laboratory post-mortem records suffers from the effects of owner selection. Owners generally attempt their own diagnosis and submit carcases only when the findings are obscure or the death rate becomes excessive.

A total of fourteen flock owners agreed to take part in this survey and after post-mortem examinations had been carried out for one year all owners were asked to provide from their own records, stock returns, numbers of losses and reasons for any discrepancy between this figure and the number of carcases received at the laboratory.

All carcases were examined in some detail. After inspection for external lesions or abnormalities the bird was nailed on a board and the contents of the abdomen and thorax were exposed by removal of the abdominal wall, sternum, most of the ribs and intercostal muscles. Any obvious lesions were noted and cultures and material for section taken from them. Heart blood was cultured in all cases. Then the internal organs were removed and examined individually. The mouth and pharynx were opened by cutting through the lower jaw on one side and the incision continued down the trachea. The skull was divided trans-

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versely to expose the sinuses and the brain was exposed. The alimentary canal was opened throughout its length and if there was any suggestion of abnormality wet smears were examined and in many cases cultures were made from the crop wall, intestinal and cæcal contents. The sciatic nerve, brachial plexus, hock and stifle joints were also examined. If intestinal nematodes appeared to be present in fairly high number a worm count was carried out. A definite diagnosis was made only when the lesions were reasonably specific or a causal agent was isolated. Doubtful cases were included under the heading of "no definite diagnosis" although many of these carcases showed well-marked changes.

Results

The information provided by flock owners at the end of the first year's survey underlined the difficulties of collecting accurate records of losses. Of the fourteen flocks originally co-operating, four had lapsed and seven of the

FIGURE 1

FARM	STOCK RETURN 1951						LOSSES	
	BREEDING STOCK	HATCHED	BOUGHT AS DAY OLDS	SOLD	STOCK AT END OF YEAR	DEATHS ESTIMATED BY OWNER 1951	CARCASES SUBMITTED FOR EXAMINATION	
							1951	1952
A	600	10198	—	8022	600	2176	1019	639
B	178	5870	—	5670	220	161	141	97
C	—	361	208 (3WK OLD)	466	—	103	76	85
D	—	—	290	192	—	98	3	—
E	—	—	1300	970	—	330	2	—
F	—	—	400	350	—	50	9	2
G	—	—	300	292	—	8	—	—
H	24	424	—	297	48	53	—	—
I	7	124	—	123	7	1	—	—
J	—	—	68	59	8	1	—	1

others had submitted 14 carcases although suffering a total loss of 540 birds. The main reasons for this discrepancy were the difficulties of getting carcases to the laboratory and the fact that in many cases the owners were satisfied with their own explanation of the cause of death.

There remained Farms A, B and C (see Fig. 1) and as these were all near to the laboratory carcases were either brought in by the owner or collected by us. These were the only farms from which a reasonably high proportion of losses was examined, but by no means all carcases were submitted. Seventeen carcases from Farm A were sent to another laboratory which made a diagnosis of erysipelas in two cases and catarrhal enteritis in the other fifteen. There were also heavy losses from foxes and a large number of deaths occurred in August (see Fig. 3) when the carcases decomposed too rapidly to be of value. The difference of twenty carcases on Farm B was accounted for by those salvaged

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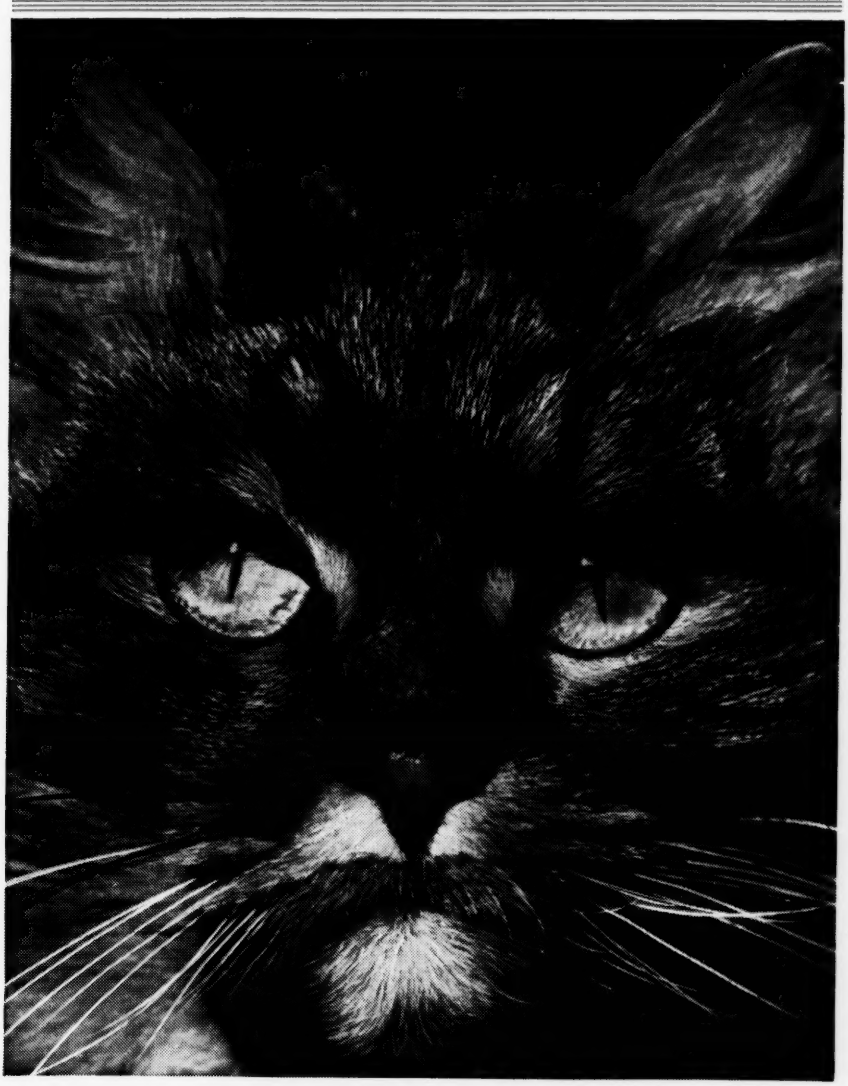

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for human consumption and those fed to dogs, whilst on Farm C the owner admitted six fox losses but claimed 20 more carcases were forwarded than our records showed.

It was clear that for several reasons it was difficult and probably impossible to survey deaths on farms some distance from the laboratory and so for the second year's survey we invited the co-operation of only Farms A, B and C. Farms A and B kept a breeding flock and practised large-scale incubation. The young poults were housed in heated brooders and gradually hardened off into open verandahs with wire or slatted wood floors. On Farm A the poults when about 12 weeks old went out to grass in small fold units moved daily, whilst on

FIGURE 2

ANALYSIS OF POSTMORTEM FINDINGS

DIAGNOSIS	FARM A				FARM B				FARM C			
	1951		1952		1951		1952		1951		1952	
	T	%	T	%	T	%	T	%	T	%	T	%
NAVEL INFECTION	82	8	59	105	118	84	41	43	5	6	1	2
GIZZARD EROSION			51	8			3	3	1			
COCCIDIOSIS	201	20	97	15			14	14			11	13
ULCERATIVE ENTERITIS	303	29	121	19	3	2	25	26	1		15	16
GIZZARD IMPACTION	90	9	9	2							9	11
BLACKHEAD	20	2	10	2	8	6	3	3	36	47	2	2
PASTEURELLA	4											
INJURY	8		38	65					3		4	
STREPTOCOCCAL SEPTICAEMIA	3											
ASPERGILLOSIS	7		7		2		1		2		4	
INTESTINAL IMPACTION	4		1									
CROP IMPACTION	5		1									
MONILIASIS	3		5								7	
PERITONITIS	1		2									
VISCERAL GOUT	1		4		1				1			
ERYSIPELAS	8		3									
ARTHRITIS	1		2		4				1			
PEROSIS	1		1						4		1	
INTERNAL HAEMORRHAGE			9						2		1	
SINUSITIS											4	
RUPTURE OF OVIDUCT			1									
NO DEFINITE DIAGNOSIS	277	27	218	30	50	3	10	10	20	26	26	31
TOTAL	1019		639		141		97		76		85	

Farm B at about 16 weeks the poults went on to free range in groups of 50 or more. Farm C bought in day-old poults, placed them in heated brooders and gradually hardened them to open slatted-floor verandahs where they remained until fat.

These farms continued to submit carcases and both years' results from the three farms are analysed in Fig. 2. This figure shows that a variety of conditions occurred, some in small numbers and others frequently. Both actual and

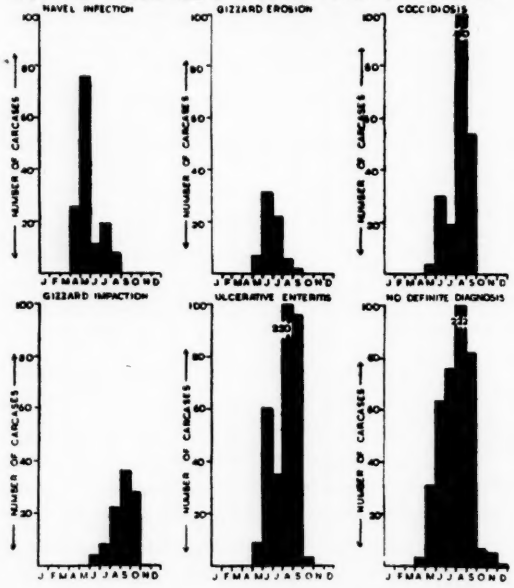
percentage totals are given for the more frequently occurring diseases, which were : navel infection, coccidiosis, ulcerative enteritis and, to a lesser extent, gizzard impaction, gizzard erosion and blackhead. Another large body came under the heading " no definite diagnosis," and the significance of this will be discussed later.

Navel infection.

This diagnosis was made on the following post-mortem picture. Poults usually 2 to 4 days old, but sometimes up to 10 days of age, either died or were destroyed when moribund. The yolk sacs were larger than normal for their age and the contents were liquid, often grey in colour, foul smelling, and many had ruptured into the peritoneum. The navel was usually moist and often covered with encrusted dirt. The livers were of bright orange colour, there was peritoneal fluid and occasionally myocardial degeneration and lung congestion. Bacteriological examination of the yolk sac gave a mixed growth of coliforms, streptococci

FIGURE 3

MONTHLY INCIDENCE OF 6 CONDITIONS ON FARM A



and staphylococci and usually the same organisms were recovered from the heart blood. Gordon (1955) describes the disease under the heading " mushy chick disease " and suggests that intestinal bacteria from the adult infect the embryo through the shell or at hatching.

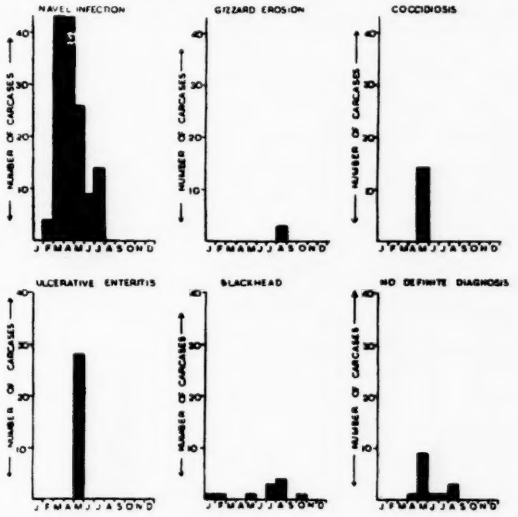
Naturally the greatest losses from this condition occurred when hatching was heaviest, during May on Farm A (Fig. 3) and during March and April on Farm B (Fig. 4). There was a small peak in August at Farm C (Fig. 5) in a batch of late-hatched poults.

Gizzard Erosion.

Although not causing particularly heavy losses, this condition is mentioned next as it appeared to occur in the poults slightly older than those affected by navel infection. It occurred mainly on Farm A with a peak in July (Fig. 3) when the poults were just over a month old. They died in thin condition with empty crops and little food in the gizzard. The horny lining of the latter was loose and under-run and large areas had been shed completely. Many investigators have associated this condition with a deficiency of an essential nutrient present in greenstuff and the incidence appeared to diminish after the addition of grass meal to the ration.

FIGURE 4

MONTHLY INCIDENCE OF 6 CONDITIONS ON FARM B



Coccidiosis.

This hazard in the poults life occured at ages varying from a minimum of 10 days up to and more commonly at the 10-12 week period. Thus on Farms A and C the peak was in August whilst on Farm B small outbreaks occurred in May in a batch of 14-day-old poults. A diagnosis was only established when schizonts and oocysts occurred in large numbers together with considerable damage to the mucosa. The commonest site of the lesion was the lower part of the small intestine, though in many cases the cæcum and large intestine were involved. The mucous membrane was severely inflamed and the intestinal contents were of a creamy-white mucoid nature. Smears of this material often contained little else but oocysts. Oocyst measurements suggested that both *Eimeria meleagridis* and *Eimeria meleagrimitis* occurred. Gordon (1955) found *Eimeria adenœides* in association with blood-stained necrotic casts in the cæcum. Such a lesion was not seen in this survey.

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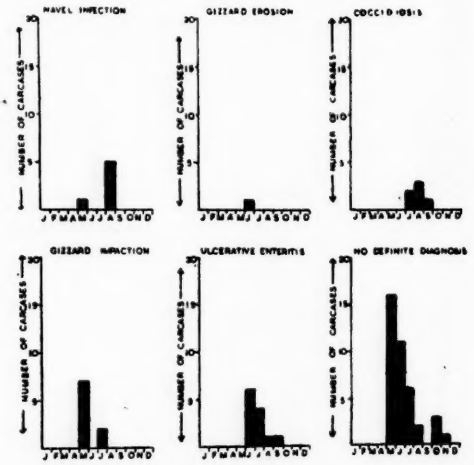
Ulcerative or Necrotic Enteritis.

This condition occurred on all farms, but its incidence was highest on Farm A and many batches of the poults were affected when 14 to 16 weeks old. Farm B had an outbreak in May in 10 to 12-week birds, and Farm C lost birds of the same age in June. The disease usually appeared suddenly in a pen of birds, several would lose their appetites, appear listless and occasionally show watery diarrhœa. Death often followed rapidly but chronic cases did occur. As no causal agent was incriminated a definite diagnosis could only be made in the presence of typical lesions in the small intestine (see Fig. 7). These were in the form of local areas of necrosis of the mucous membrane which took on a greyish-white colour and when extending deep into the mucosa the lesions were visible through the serous coat of the unopened intestine. Microscopically the loss of

FIGURE 5
MONTHLY INCIDENCE OF 6 CONDITIONS ON FARM C



structure and staining affinity was seen to extend to various depths in the mucosa. There was no definite leucocytic zone at the junction of necrosed and normal tissue and any accumulations of bacteria were near the surface of the necrosed mucosa rather than in one well-marked zone at the junction with the normal tissue beneath. This suggested that any bacterial invasion occurred after the necrotic process and that the primary cause of the latter was not a bacterial invasion. It was possible to scrape away the necrosed mucosa in the form of small, coherent masses, but as the lesions were round and discrete, varying in size from a pinhead to $\frac{1}{4}$ in. diameter, a continuous diphtheritic membrane never occurred. At the same time that these lesions were occurring, other dead birds were examined and showed hæmorrhagic or mucoid enteritis in varying degrees, but in the absence of an obvious cause they were classified as "no definite diagnosis."

In view of heavy losses associated with necrotic enteritis, many efforts were

made to detect the cause. Intestinal smears, both wet and stained by Gram, Leishman, Giemsa and Field's methods, were examined from recently-dead or freshly-killed birds, but no *Hexamita* or *Trichomonas* were found, nor were any protozoa consistently seen except that oocysts occurred in some cases, but these were so few in number and the lesions so unlike those of coccidiosis that they were not considered to play a causal role.

Many bacteriological and histological examinations were carried out. In a number of cases *Clostridium welchii* Type A was recovered from the intestinal contents. This strain was grown in a cooked-meat medium and two 7-week-old poults were given 5 ml. of the culture by mouth and two similar poults were injected rectally with the same amount. In addition, a stock strain of *Cl. welchii* Type A was used on four other birds. All eight birds remained healthy and were killed eight days later to show no lesion whatever.

It was still felt that an infectious agent might be incriminated, and further transmission experiments were carried out.

The intestines from a sick bird which had been killed and had shown typical lesions were removed and minced finely in normal saline. A portion of the material was passed through a Seitz EK pad and one 6-week-old bird was dosed orally with 3 ml. of the filtrate and a second 6-week-old bird was given an intramuscular injection of 2 ml. in the breast. These two birds showed no signs of illness and there were no lesions when their carcases were examined three weeks later.

It was possible that the filtration had removed the infectious agent and so further attempts at transmission were made. The crude suspension of minced intestines was filtered through coarse muslin and two more poults of the same age were given an intramuscular injection in the pectoral region of 2 ml. Twenty-four hours later both birds died with a rapidly-spreading necrotising lesion of the breast. There were no intestinal lesions. Two other poults received 5 ml. of crude filtrate by the mouth; one remained healthy and the other died seven days later with typical intestinal lesions. Bacteriological examination of the heart blood and the intestinal contents produced no pathogenic organisms, whilst histological examination of the small intestine showed the typical picture previously described. Two other birds dosed with 5 ml. of crude filtrate died two and six days after dosing. Both carcases had empty crops and gizzards and congestion of the rectal mucosa. There were no visible lesions of the small intestine and heart-blood cultures were negative. Two other birds were kept in adjoining cages to the dosed birds and one of these was found dead seven days after dosing. The crop and gizzard were empty and there was a hæmorrhagic enteritis and typhlitis. Smears of the contents showed nothing significant and heart-cultures grew coliforms only.

Further attempts at transmission were carried out with minced intestines from later cases. Of two poults given 20 ml. of crude material by mouth, one remained healthy and the other died 14 days later with no intestinal lesions. The intestines from the second bird were minced and 10 ml. was given by mouth to one 14-day-old poult; there were no ill effects.

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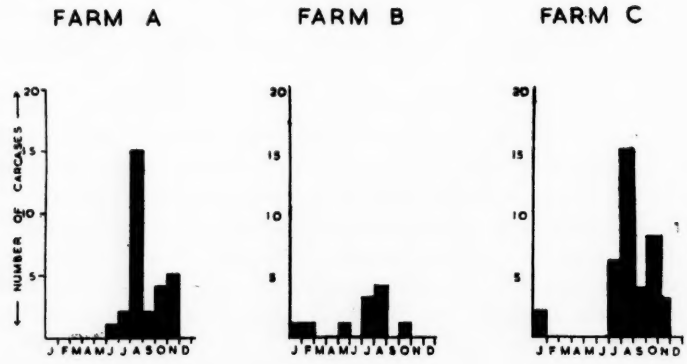
These attempts at transmission may be summarised by stating that intestines or their filtrate from four cases were given by various routes to 16 poults. The filtrate was innocuous and intramuscular injection of unfiltered material killed rapidly with a severe local muscle necrosis. Of 12 poults dosed orally or rectally six died in one to fourteen days and one of these showed typical lesions. One of the in-contact birds also died showing a marked hæmorrhagic enteritis.

Gizzard Impaction.

This was a simple mechanical obstruction of the gizzard, proventriculus and in many cases the first part of the duodenum with tangled grass fibres or fibrous litter. The peak incidence was during September on Farm A and during May on Farm C. Here the obstruction was caused by peat-moss litter in 6-week-old poults. The condition did not occur on Farm B, where poults remained on wire-floored verandahs until a later age than those of Farm A.

FIGURE 6

MONTHLY INCIDENCE OF BLACKHEAD



Blackhead.

Farm B had a low incidence of 4 per cent and cases were distributed throughout the year (see Fig. 6). Farm A had an incidence of 2 per cent, with a peak in August when the poults were about 12 weeks old. Farm C had a serious outbreak in the first year of the survey commencing in July, with a peak in August, and carrying on until November, when most birds were slaughtered. The birds were housed in open wire verandahs and fed on rations which did not include green food. Water was provided in continuously-flowing troughs fed from an automatically-emptying cistern. An attempt was made to medicate the water with sodium acetarsol, but there is no doubt that a proper concentration was not maintained continuously owing to the nature of the water supply. After lengthy inquiries a fairly reasonable explanation of the outbreak was discovered. The building in which the turkeys were housed was a modern multi-purpose

structure containing, on the ground floor, turkey verandah, incubator rooms and cockerel-fattening battery. On the first floor were hen batteries, deep-litter house, food store and mixing floor. The exit from the deep litter was over the floor on which the turkey food was mixed and it seemed likely that the boots of the attendants coming from the deep litter could contaminate the turkey food. On investigation it was found that many of the hens on the deep litter were infected with *Heterakis* worms and it was later shown that *Heterakis* eggs from this source were capable of setting up Blackhead in poults. When food mixing was carried on in another place during the second year's survey the incidence of Blackhead in the poults was much lower.

No Definite Diagnosis.

This heading covers a large number of carcases received from all three farms. It includes a number of dead birds showing lesions not sufficiently specific to allow classification under a definite heading. Many of these cases showed intestinal changes varying from slight congestion to catarrhal enteritis, and it is very likely that the majority were early cases of what would have been classified as ulcerative enteritis had the lesion developed to a later stage. This suggestion is supported by the fact that the peaks for ulcerative enteritis are similar to those for conditions not definitely diagnosed.

Discussion

This investigation was initiated because it was felt that the common impression that Blackhead was the major disease of turkeys might be erroneous. Although investigations were commenced in 14 flocks, useful figures were obtained from three farms only. This experience confirmed the well-known difficulties encountered in all attempts to obtain accurate information of losses due to disease. In the three flocks surveyed, Blackhead was the cause of negligible loss in two, whilst losses were greater in the third and were traced to an error in management.

Total losses in these three flocks were 2,440 out of approximately 16,600 poults hatched or bought in the first survey year. The main causes of loss were navel infection, gizzard erosion, gizzard impaction, coccidiosis, ulcerative enteritis and a large group of undiagnosed cases, many of which were probably atypical ulcerative enteritis. Many other well-recognised diseases of turkeys occurred, but cases were few in number, although these figures do not necessarily represent the true incidence. For example, the erysipelas and pasteurella outbreaks on Farm A occurred late in the year in pens of turkeys that were in fat condition, and as soon as the diagnosis was confirmed the rest of the birds were killed, thus bringing to an end what might have been, had they occurred in younger birds, outbreaks of serious proportions.

Again, the number of cases of a condition which occur will vary according to the efficiency of methods of treatment available. For example, in the early stages coccidiosis mortality was high, for although Sulphadimidine in the drinking water was offered to each hatch as soon as diagnosis was clear, the birds did not take readily to the medicated drinking water. Later, sulphaquinoxaline

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treatment was substituted and the duration of each outbreak was shorter. Infectious sinusitis is another example of a disease of low mortality but high morbidity. In spite of a widespread outbreak on Farm C, the total deaths from this disease were only four.

On the other hand, the figures for gizzard impaction give a true picture of its incidence on Farm A. It was known that deaths commenced in each hatch soon after they were put on grass and could be prevented by housing the birds in verandahs, yet the incidence was such that it was not considered an economic proposition to change the husbandry to avoid this small loss.

Gordon (1955) examined 600 turkey carcases over a period of 18 months. Four hundred of these carcases were from one farm where the two commonest diseases were salmonella infection and sinusitis. The remaining 200 cases consisted of 36 per cent of bacterial origin, 38 per cent of protozoal origin, 5 per cent fungal infection and 8 per cent nutritional deficiencies. The four most numerous individual groups were 21 per cent salmonellosis (mainly *S. typhimurium* infection), 20 per cent blackhead, 15 per cent coccidiosis, and 10 per cent sinusitis. Dearstyne (1945), surveying losses in one flock, found 32 per cent blackhead, 19 per cent pendulous crop, 10 per cent mycotic infections, 10 per cent reproductive disorders and a variety of miscellaneous conditions. Bullis (1945) examined 2,500 carcases in ten years and found coccidiosis, blackhead, salmonellosis and ulcerative enteritis to be important causes of loss.

Thus all three investigators find blackhead, coccidiosis and salmonellosis to be important conditions.

To return to the present survey, apart from the category of conditions not definitely diagnosed, those cases classified as ulcerative enteritis formed the most important category numerically and in spite of extensive diagnostic efforts the ætiology of this condition remains obscure.

Many workers have described outbreaks of enteritis in turkeys. Bullis (1945) mentions both enteritis and ulcerative enteritis, so it would appear that he also encountered an entity with a necrotic lesion. He makes no comment as to the ætiology of the condition. Campbell (1945) described lesions in 2- to 4-week-old birds which had a congested intestinal mucosa and yellowish, frothy intestinal contents. Protozoa of the genus *Cochlosoma* were found in the intestines, but no transmission experiments were attempted. Doyle, Cable and Moses (1945) found similar macroscopic lesions to those of Campbell. The intestines yielded a variety of flagellates including *Hexamita meleagridis,* but the latter did not occur constantly and could also be passaged without causing characteristic symptoms. Hinshaw, McNeil and Kofoid (1938) had discussed the relationship of *Hexamita* to turkey enteritis and concluded that the parasite was capable of causing a specific enteritis with lesions similar to those described by Campbell and by Doyle *et al.*

All the published accounts of *Hexamita* infection describe a catarrhal enteritis quite distinct from that designated here as ulcerative enteritis and repeated examination failed to detect *Hexamita* in association with the latter condition.

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A disease occurring in quail and macroscopically resembling very closely the condition described as ulcerative enteritis in turkeys was described by Bass (1934). He was able to passage the disease by feeding macerated intestines or pure cultures of a Gram-negative bacillus isolated from the lesions.

Our investigations into the causation of ulcerative enteritis did not produce any conclusive results. The transmission experiments resulted in a number of deaths, one of which showed diagnostic lesions. It is possible that an infectious agent was present, but the optimal conditions for its passage were not reproduced in these experiments. Gordon mentions that a condition is recognised in the U.S.A. which affects growing poults and can be transmitted by unfiltered intestinal contents.

Summary

An attempt was made to investigate losses in 14 turkey flocks, but due to various difficulties figures were finally obtained from three flocks for a two-year period. The mortality was 10 per cent, 2.6 per cent and 13.3 per cent of poults hatched, and the important causes of loss were navel infection, coccidiosis, gizzard impaction, gizzard erosion, ulcerative enteritis, and a large category of undiagnosed cases. Attempts to throw light on the cause of ulcerative enteritis gave indefinite results, but there was a suggestion of a transmissable agent.

Acknowledgments

I am grateful to Mr. D. L. Hughes, F.R.C.V.S., Dip.Bact., for suggesting the investigation, to Miss J. Newell and Miss J. Stephenson for technical assistance, and to the Directors of Messrs. Boots Pure Drug Co. Ltd. for facilities.

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VIBRIOSIS IN DAIRY CATTLE WITH REFERENCE TO THE TREATMENT OF A PARTICULAR OUTBREAK WITH STREPTOMYCIN SULPHATE (STREPTOQUAINE)

By J. B. TUTT, M.R.C.V.S.

Winchester

Introduction

VIBRIOSIS is one of the most important causes of infertility in dairy cattle in this country. This article describes the symptoms as seen by the author in several confirmed outbreaks of a serious nature in the county of Hampshire. Special reference is made to a form of treatment carried out fully in one infected herd.

In order to present my remarks in a logical order many observations will have to be made that have been reported already by other workers. This is unavoidable for although my observations started in 1949, I have not published any account thus far. Much knowledge on this condition has been accumulated and published. Except for the bacteriological descriptions and where otherwise acknowledged, the rest of the findings in this report have been observed by the writer personally and in the course of general practice.

Ætiology

The organism is known as vibrio fœtus, an S-shaped, gram negative organism with delicate growth requirements. In order to isolate it from infected material, this material must be in a fresh condition, as the organism is easily swamped by other bacteria. It stains with carbol fuchsin; in fresh material appearing in pairs, giving a " flying seagull " appearance. Roberts (1950) states that cattle under one year old are resistant. Transmission is believed to be mainly venereal, via a carrier bull or cow.

Symptoms

The symptoms to be described are taken from a series of outbreaks. While most of the clinical signs will be seen in a typical outbreak, it is not suggested that the clinical picture will be clear-cut and defined in each case.

There will be a herd infertility problem with over 50 per cent of the cattle returning to the first service. If the stock bull is used on the heifers, as used in the main herd, these animals will be found to be returning to service. Although a few animals will return at regular periods, the majority will be found to be returning at irregular periods, i.e., 25 to 39 days being a common time. Sometimes the outbreak can be traced to the admission into the herd of a bought-in cow or bull. If the service dates are examined chronologically, such an animal can be sometimes found. In other cases, it has not been possible to come to a conclusion as to the origin of the outbreak.

An early symptom in the female is a vaginitis a few days following infected

service, but this is slight and transient and resolves easily and may not be noticed. Infected animals at subsequent œstral periods sometimes show a clear discharge, which is in greater quantities than seen normally. The amount of this discharge has been so pronounced in one herd that the owner commented upon it. The bull usually shows no symptoms; it has been stated that a transient inflammation of the penis and the lining of the prepuce occurs. This condition has not been observed.

When the disease has been established for some time, retained placentæ, occasional abortion, and the birth of weakly calves are clinical features. The abortions tend to occur about the fifth month of pregnancy: cases have been encountered at three months and eight months, all of which were positive to vibriosis. It has been stated that aborted fœtuses from infected animals may be bacteriologically clear. Some infected animals will calve normally to time but be difficult to get in calf subsequently. The abortion rate is not so high as in Brucellosis. Plastridge quotes an average of 12 per cent with a range of 4 to 20 per cent.

The initial infertility in a recent outbreak eventually resolves itself to a greater extent, as the majority of cows will hold after repeated services; these average 6 to 8 in number. Should the stock bull continue to be used on the heifers, the infertility will be ever-present in this new batch of animals each year. This wastage of service time causes great economic loss to the farmer, due to loss of milk sales.

It would appear that the main spread is venereal; it has not been possible to implicate ingestion as a route of infection in the cases seen. This method of spread may exist as a potential factor but in my opinion is not the main method.

Methods of Diagnosis

Clinically, a history of herd infertility showing the features already described. Confirmation is by laboratory methods and the following were employed:

(*a*) *Examination of Vaginal Mucus for Agglutinins.* This method was employed as a herd test, one positive result from a number of samples being taken as evidence of infection in the herd. Mid-œstral period was the most favourable time, with the œstral period being the most unfavourable time to collect samples. When a herd was sampled, it was not possible to choose this favourable period in all animals. Therefore, as large a number of animals as possible were tested; as a result, a number of negative individual reports were obtained, but in suspected herds, positive results were found, provided a reasonable number of samples were taken, and that these samples were adequate for the laboratory. This was the main method employed to arrive at a definite diagnosis, and it proved to be very satisfactory.

(*b*) *Examination of the Aborted Fœtus.* An aborted fœtus must be sent by road to the laboratory as the organism is so fragile that delay causes it to be swamped by other bacteria, and examination is useless. Fœtuses from five herds were so examined and four were found to be positive. In others a negative report was received. As it has been stated, some fœtuses are reported

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negative to vibrio fœtus although from an infected animal or herd proved to be infected with vibriosis by other means.

(*c*) *Examination of Fœtal Membranes.* Recently at the suggestion of Mr. S. Hignett, of Frant, the placentæ have been sent from a number of consecutive parturitions in a herd in which other methods had failed to confirm a diagnosis. In one herd there had been no abortions; it had proved difficult to obtain good vaginal samples and confirmation of infection had not been obtained, although the clinical picture of the herd was very suggestive of vibriosis. A positive result was obtained by this method. Mr. S. Hignett has stated that placentæ from twelve consecutive parturitions, whether normal or not, should be examined before a herd can be declared free of the disease.

(*d*) *Examination of Bull's Semen.* This method has not proved satisfactory and is not practised now. In one herd, vibrio were isolated from the semen of a bull. Subsequently an animal served by this bull aborted at four months, and examination of this fœtus was positive to vibriosis. A better method for bull testing would be for them to serve virgin heifers whose record could be checked later and mucus samples taken from them. Up to date this method has not been used by me.

Treatment of an Outbreak

The first essential is to divide the herd into two parts: (*a*) the clean herd consisting of unbred animals and (*b*) the main infected herd. The object of this grouping is to build up a clean herd irrespective of whatever other lines of treatment may be adopted. This segregation must be strictly adhered to by all workers in the dairy with regard to service, as a slip on their part would lead to disaster. The clean herd should have a fresh virgin bull or be A.I. The latter is preferable as bought-in bulls must be always regarded with suspicion. These have often been tried before sale on animals to see if they are capable of service or may have only "played" with other cows. Nevertheless, this may have been sufficient for them to have picked up infection.

When the heifers come into the main herd, this sexual segregation must be adhered to strictly. This will enable a disease-free herd to be rebuilt.

The following methods of treatment can now be tried on the infected herd:—

(*a*) A sexual rest of three months is said to have beneficial results. The cows either acquire a tolerance or free themselves from infection. Possibly both may occur in a particular herd. This method has not proved very satisfactory in the herds under review. The rest causes economic loss to the farmer and in five herds they were not willing to carry out this plan. This is the main disadvantage as the owner feels that nothing is being done and should the method prove unsatisfactory there is a loss of confidence on the farmer's part. This method was tried in one herd where an average of six months' sexual rest was given. At the end of this period the greater number of animals still did not hold to A.I and as a result some 30 cows were sold as barren. Reports from other veterinary sources, however, indicate that this method has produced quite good results.

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(*b*) The policy of unrestricted service was carried out in about four herds. It was found that after about six to eight services the majority of the animals eventually became pregnant, and most of them held to subsequent service after parturition.

There were certain disadvantages to this practice. These were:

(1) Loss of time in getting the animals pregnant.

(2) There was a danger of a secondary infertility developing in the stock bull, if only one was in use, due to overwork.

(3) It is more likely to propagate a chronic infection in the herd which is liable at a later date to become acute.

Treatment with Antibiotics

Lofthouse (1951) describes an outbreak where penicillin injections proved highly successful.

After a private discussion with Mr. S. Hignett, it was decided to try streptomycin. In some of the herds a pleuropneumonia-like organism had been isolated. Its significance could not be assessed at that time. It was sensitive to streptomycin, and this was why this antibiotic was chosen. In actual fact, in these herds where streptomycin was eventually used, this organism was not isolated.

In one selected herd streptomycin in two ounces of distilled sterile water was introduced into the uterus on five successive days in a selected number of animals. Twelve such animals were treated and nine held to the first service after treatment. This appeared fairly satisfactory and it was decided to give the antibiotic by intramuscular injection, as it was an easier method of treatment and did not involve injecting fluids into the uterus. The dose employed was four grams as the initial dose, followed by two grams at 24-hourly intervals for four successive days. The injections were given in the fourteen-day period before service. The bulls were to have a similar course of treatment, and this in their case was to be repeated every three months to guard against re-infection.

Results were as follows:—

Number of animals holding to first service after treatment—9.

Number of animals holding to service just prior to treatment—9.*

(* These animals had returned to service repeatedly; as they had just been served prior to the start of the investigation it was decided to inject them.)

Animals holding to the second service after treatment—6.

Animals treated but not pregnant—7.

The animals which are noted as holding were confirmed as pregnant.

The average number of services per animal prior to treatment was 4.5.

Although these experiments cannot be regarded as being fully successful, there was a definite improvement in fertility.. It was on the results of this preliminary investigation, which was carried out under the guidance of Mr. S. Hignett, that the specific treatment of the following outbreak was initiated.

The herd was a pedigree Ayrshire herd started in 1944 by buying in cows and bulls. It was first investigated as having an infertility problem in the autumn of 1950. This herd then consisted of 44 milking cows and two bulls.

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One bull was hired in December, 1949, and returned in July, 1950. It is interesting to note that most of the confirmed outbreaks of vibriosis in my practice started in this period. The infertility in this herd had commenced in the spring of 1949, so the above-mentioned bull was not the originator. Normally the cows were served at the third postpartum œstrus. In 1949 20 out of the 35 cows returned to the first service. Out of these 20, more than 50 per cent returned to a third service. In 1950. 25 cows returned to the first service out of 44, and of these, a large number returned several times. There was a slight increase in retained placentæ, followed in a few cases by a flaky uterine discharge. The heifers had calved down normally with good udders. There had been no increase in the milk yield following intra-uterine injections of iodine solutions. A calf's thyroid, which was sent away to the laboratory, was examined for an iodine deficiency with a negative result. The herd was having regular S 19 vaccinations given to the calves. In 1950 there had been two abortions, one of which was positive to Brucellosis.

A detailed examination of the herd was carried out, as follows:

(*a*) Semen samples were taken from the bulls and were found to be of normal fertility.

(*b*) Examination of cows which had calved about 28 days previously showed that in a number of cases, normal involution of the uterus was incomplete.

(*c*) Several animals at different stages in the œstral cycle were examined and in a number of cases the corpora lutea were ill-formed. In one case there was cystic degeneration.

(*d*) Vaginal samples were taken but were unsatisfactory and yielded negative results.

(*e*) The pH values of the fields grazed were from 5.9 to 6.9.

No placentæ were sent away at that time as this method of diagnosis had not been considered. No fœtus was available for laboratory examination. The ration details were examined, and samples of the foods used were sent away for chemical analysis to ascertain the P_2O_5 and CaO levels. This revealed a low phosphorus level and a wide Ca/P ratio. As a result of this investigation the owner was advised as follows: Although there was no bacteriological evidence that an infection was present, its existence should be considered, and secondly, that the nutrition of the herd was at fault. To correct the diet it was suggested that a phosphorus high mineral mixture should be fed together with fish meal. Instructions were left that any fœtus aborted should be sent by road to the laboratory.

Following these recommendations, there was a distinct improvement in the fertility rate although a number of animals returned repeatedly to service. Further consultation was not requested on this subject until February, 1954, when the problem had become acute. On looking into the breeding records between 1951 to 1954, it was seen that the initial improvement in the fertility had declined in the years, becoming worse latterly. The position had suddenly become acute about Christmas, 1953, when a large number of animals went

to service and started returning. This was the position when the herd was re-examined in February, 1954. Details will now be given.

At this date the herd, an attested Ayrshire herd vaccinated against Brucellosis, consisted of 42 cows, 16 bulling heifers and three bulls. A study of the breeding records from September, 5, 1953, to February 17, 1954, revealed these details:

September	...	Animals served for the first time				5	Animals re-served		3
October	...	,,	,,	,,	,,	4	,,	,,	7
November	...	,,	,,	,,	,,	2	,,	,,	8
December	...	,,	,,	,,	,,	13	,,	,,	10
January	...	,,	,,	,,	,,	12	,,	,,	16
February	...	,,	,,	,,	,,	6	,,	,,	5

Three bulls were in use, and switched as animals returned to service. The clinical history revealed the following features:

(*a*) Return to service was irregular, averaging about 35 days with a range of 14 to 63 days.

(*b*) There had been no noticeable uterine discharge.

(*c*) Four animals had retained their placentæ in 1953.

(*d*) There had been no abortions. Later there were three abortions, and two of them were positive to vibriosis.

(*e*) One cow had been bought in 1955 but there was no connection between this animal and the acute condition now present.

(*f*) Subœstrus was not a feature. The heifers were returning to service as frequently as the cows. The stock bull was being used on them.

(*g*) The maintenance and production rations were on the lines suggested at examination in 1950. The water supply to the farm was hard.

(*h*) The cows were grazed on ley pastures consisting of mainly cocksfoot and white clover with a little timothy. Nitrogenous fertiliser was applied each spring at two cwt. per acre. A mixture of superphosphate and potash was added annually, before the nitrogenous fertiliser, at the rate of three cwt. per acre.

This completed the history of the herd and the animals were then examined. They appeared in good condition with good coats and skin; there was no evidence of a copper deficiency. A group of animals which had been in season 7 to 14 days were examined. The corpora lutea were well formed. The uteri of animals calved about 28 days previously were found to have involuted normally. There was no evidence of a low œstrogen level. There was no evidence of a uterine discharge or vaginitis.

A group of animals which had been in season 72 hours previously appeared to have ovulated normally. Amongst the few animals examined which were

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supposed to be about 9 to 16 weeks pregnant, no case of pyometra was found. This suggested that the infection was not Trichomoniasis. On examination it was found that only seven dairy cows were pregnant whereas 35 were not pregnant. Out of 16 heifers only two were in calf.

Semen samples from the bulls were of normal fertility and no pathogen was found. Vaginal samples were taken from 20 animals, 12 were found to be positive, four doubtful and four negative to vibriosis. No pleuropneumonia-like organism was isolated from six samples, or from the semen. This confirmed the clinical diagnosis of vibriosis.

Method of Control and Treatment

The owner was advised of the various available methods. It was decided to segregate the herd for service as previously outlined, and to use A.I. on the clean herd. He was instructed to send away any aborted fœtus for examination. Three occurred, and, as stated, two were positive to vibriosis.

It was decided to raise the herd resistance to Brucellosis by revaccination of the dairy cows and to repeat this every three years. Streptomycin sulphate in the form of Streptoquaine was chosen as the antibiotic for individual treatment. All animals in the infected herd were to be treated by injection into the gluteal muscles in the three-week period prior to service. The bulls were to receive similar treatment, which was to be repeated every three months. The dosage taken was that already quoted, a total of 12 g. per animal. The animals already pregnant were to be treated prior to re-service after calving. After treatment, some animals were to be served by the bulls and some by A.I. The injections did not cause any local or systemic reaction, nor were the bulls upset by the repeated injections. The results were as follows :—

Thirty animals, excluding the bulls, were treated; 24 held to the first service after treatment and on examination were pregnant. Of the remaining six animals, after a second course of injections, five held to subsequent second service and three were confirmed as pregnant. One animal was still barren.

The bulls were used on 13 cows; only two returned and these two animals held to the next service. A.I. was used on the remaining animals, four returning, of which three appear to have held on re-service.

Observations

(1) Vibriosis was probably present in the herd in 1949, together with a nutritional imbalance. When the animals were examined in 1954, the state of the ovaries and uterus was indicative that this imbalance no longer existed. Nevertheless, an acute flare-up had occurred in 1953-54. Some other factors had come into operation to cause this problem. On surmise, it would appear to be either an increased virulence of the organism and/or lowered bodily resistance of the animals. The bulls were of normal fertility, and the clinical examination of the herd did not suggest any deficiency of a definite or induced nature.

No other pathogenic organism had been isolated from the semen or vagina.

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(2) The spread was venereal. Taking into consideration the fragility of the organism, it is so easily swamped by other bacteria, it is unlikely to be able to exist for long in outside environment. The spread from ingestion could almost be ignored.

(3) The use of streptomycin in the doses employed produced very good results in obtaining pregnancy. How many will abort is not yet known; nevertheless, the initial obstacle has been overcome, that of infertility. It would suggest that the infection has been cleared, as insufficient time has elapsed to acquire an immunity. The state of the bulls will be difficult to prove; they are to have the repeated treatments as they are exposed to reinfection by service. Test mating would have to be carried out to see if they are clear. The time is not ripe to carry this out on the herd.

Summary

A general description of vibriosis is given, arising from the investigation of the condition in several herds in Hampshire.

The various control methods which are at present in vogue are discussed.

Particular details regarding history, clinical findings and treatment with streptoquaine of a herd are described in full.

The results are suggestive that this antibiotic is of some practical use in infected herds, provided that the nutritional requirements of the herd are also corrected.

Acknowledgments

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MURINE EAR MANGE: THE CONTROL OF *PSORERGATES SIMPLEX* INFESTATION

By ROBERT COOK

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DURING September, 1954, a large number of male and female breeding mice (Porton strain, see Cook, 1953), in each of two colonies, were found to have a "mange-like" condition of the ears. The causal parasite was identified as *Psorergates simplex,* first described by Tyrrell (1883), who found it associated with a mange occurring on the inside and outer edge of the ear of a wild mouse. This form of murine mange is not common amongst mouse stocks in this country.

Our first attempts to treat the condition with acaricides in common use were unsuccessful.

The purpose of this paper is to describe the condition caused by *Psorergates simplex* and to record our observations on the value of dibutyl phthalate as an acaricide against this parasite.

Clinical Picture and Diagnosis.

Characteristically the lesion is a thick, pale yellow crust attached to the inner and outer surfaces of the ear. In severe cases, the cream-white crust covering both ears was up to a millimetre in thickness (Fig. 1). Mechanical removal of this crust tore the skin, leaving a bleeding surface. In less severe cases lesions were confined to the outer or inner surfaces of one or both ears. The earliest cases were found amongst 12-week-old mice and these had originated from affected parents. The lesions in these cases were small, and could easily be overlooked unless a hand lens was used.

Mites, nymphs and ova were easily detected by microscopic examination of a "teased out" portion of the scab, mounted in 5 to 10 per cent KOH (Fig. 2). That the mites were living could be demonstrated by warming a piece of scab for five minutes on a hot plate kept at 40° C., then examining the specimen with direct illumination under the dissecting microscope. Mites were seen to cross the microscopic field whilst others burrowed into and out of pieces of scab.

Treatment.

Agent: Tetraethylthiuram monosulphide. A 25 per cent alcoholic solution of this drug, diluted with 14 parts of water (v/v), is effective in the treatment of rabbit ear mange (*Psoroptes communis* var. *cuniculi* and *Chorioptes communis*), and is curative in cases of murine mange caused by *Myobia musculi.* In vitro, *Psorergates simplex* mites were killed by being in contact with the diluted drug.

Twenty mice, all having severe ear mange, were divided into two equal groups. One group was totally immersed in the diluted drug. The other group had the same fluid installed into, and spread around, the ears.

Live mites were present in the ears of all treated mice at seven and 14 days after treatment. There was no better response when five animals from each group were re-treated.

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Agent: D.M.C. (di(-p-chlorophenyl) methylcarbinol). This substance occurs as a wax-like product dispersable in alcohol. It has been reported by Stoner *et al.* (1953) as being effective when used at a concentration of 0.2 per cent (w/v) in 50 per cent alcohol, against murine mange caused by *Myocoptes musculinis*. This was the concentration used in our observations.

Forty mice, all with severe ear mange, were divided into two groups of 20 mice. Ten from one group had the scabs removed from their ears. All 20 animals were then completely immersed in the D.M.C. alcoholic solution. Five mice from each of these sub-groups were re-treated in the same way 10 days later.

Live mites were still present in the ears of all 20 mice a few days after the second treatment.

The second group of 20 mice were treated by local application of the D.M.C. solution on the affected areas, 10 having the ear scabs removed before treatment. Five mice from each of these sub-groups were re-treated 10 days later. Live mites were still present in the ears of all 20 mice six days later.

Agent: Gamma isomer of benzene hexachloride. A 5 per cent (w/w) solution in miscible oil was diluted one part to 49 parts of water (v/v).

Twenty mice with severe ear mange were treated in two equal groups. One group was totally immersed in the dilute solution; the other had this applied locally to the affected areas. The treatment was repeated in all cases at 14 and 28 days after the first treatment.

Dipping mice in the diluted fluid had fatal consequences, one dying after the first dipping, two after the second dipping and the remainder following the third treatment. Ear scabs with live mites were present in all these mice up to the time of death. The 10 mice treated locally suffered no apparent ill effects but scabs and live mites were still present 17 days after the last application of fluid.

Agent: Dibutyl phthalate [$C_6H_4(COOC_4H_9)_2$]. A colourless, oily liquid, it was extensively used by Australian troops in New Guinea to combat, by impregnation of clothing, the chigger mites which were spreading scrub typhus (McCulloch, 1946).

Two mice with severe ear mange were treated locally with undiluted dibutyl phthalate, one mouse having the scabs removed before treatment. The liquid was applied with a camel-hair brush; it had good spreading and penetrating powers. On examination of both mice four days later no live mites were found. The scabs were soft and easily removed by forceps from the ears of the mouse where the scabs had been allowed to remain. Removal exposed a surface which obviously was healing.

Fourteen mice which had been treated unsuccessfully with D.M.C. were re-treated with dibutyl phthalate applied locally. On examination a month later all were found cured, scabs being almost entirely absent, and no live mites were found.

An examination of all our mice disclosed 301 cases of ear mange. One application of dibutyl phthalate cured 296, and the remaining five responded to a second treatment.

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As a preventive measure, for six months following the above observations each mouse selected as a potential breeder was treated at weaning by having a drop of dibutyl phthalate brushed into and around both ears. Not one fresh case occurred during this time, nor were there any further cases during the three months after the above prophylaxis ceased.

Discussion

The efficacy of dibutyl phthalate in curing *Psorergates simplex* mange is of interest. As has already been stated, it was used in the field by the Australians to combat chigger mites by impregnating clothing, but it is not widely used to combat disorders caused by acari. Our findings suggest that it may have a wider use than hitherto. McCulloch (1946) found it caused no adverse reactions when applied to the skin of humans over a long period of time, though it was apparently concerned with eye damage when, on one occasion, it was allowed by accident to enter the eye.

Failure of the other agents used—gamma isomer of benzene hexachloride, D.M.C., and tetraethylthiuram monosulphide—is of note as all three are of value in curing other conditions caused by mites. Such findings, have, however, been reported by others. Thus Scott (1949), using the gamma isomer of benzene hexachloride, failed to eradicate a *Psorergates ovis* infestation in sheep, and Whitnall, Thorbunn, Whitehead, McHardy and Meerholz (1949) described a gamma isomer of benzene hexachloride resistant tick.

The dipping of mice in an alcoholic suspension of D.M.C. was not only without value but also dangerous; a few dipped mice suffered permanent eye damage.

The origin of the mange in the two mouse colonies remains unknown. Observations have shown that the development of demonstrable lesions is slow. Gross macroscopic infestations have not been seen in mice younger than five to six months old. Thus the first mouse seen had probably been first infested some six months previously. The finding of many cases distributed throughout our breeding stocks suggest a common agent introduced to many mice at the same time. This is not, however, very feasible. It is doubtful if the mite ever strays very far from the area of predilection and it is hardly likely to have been introduced in such numbers, either in food or bedding, as to cause mange in about four hundred mice, many of whom had never been in close contact. It seems more probable that at least one mouse was infested when, in April, 1953, 10 pairs of young mice were chosen from the existing colony to be the basis of new colonies. However, the possibility that wild mice introduced the parasite cannot be ruled out, but undoubtedly the disease spread slowly throughout the stocks without being grossly evident for some 18 months.

Summary

An outbreak of ear mange in two mouse colonies is described. The causal agent was a mite, *Psorergates simplex*.

The disease was cured and eradicated by the use of dibutyl phthalate. Three other agents used were without value.

Fig. 1
A severe case of ear mange.

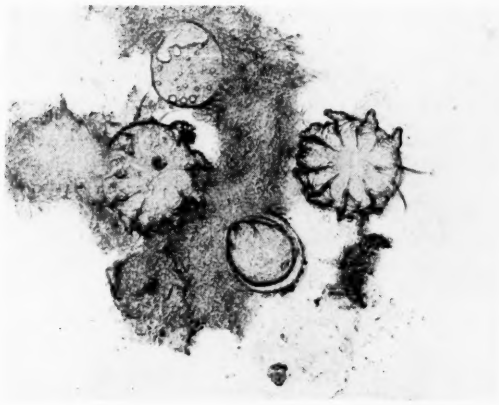

Fig. 2
Psorergates simplex—from a piece of crust mounted in 10 per cent KOH.
Photomicrograph (x 158)

(*Article by Cook, page 22*)

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Acknowledgments

Acknowledgment is made to Mr. P. Bracey, who suggested the use of dibutyl phthalate, and to the Chief Scientist, Ministry of Supply, for permission to publish this article.

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"PYOMETRA" IN THE BITCH

(With Special Reference to the Pathology of the Condition)

By J. C. WHITNEY, M.R.C.V.S.
Tonbridge

THE bitch is subject to a condition, with variable clinical and pathological manifestations, which is generally known as "pyometra." The term, which implies an accumulation of pus in the uterus, does not describe accurately the pathological changes present in all instances. In some cases there is no accumulation of fluid in the uterus and in others the fluid which accumulates is not pus. Essentially, pyometra is a condition related to hyperplastic changes in the endometrium which arise as a result of a hormonal disturbance: It has been designated incorrectly as chronic or cystic endometritis. A more apt title would be chronic subinvolution.

Ætiology

There has been much discussion regarding the significance of bacteria as a cause of pyometra. Some have considered that bacteria were the primary aetiological factors while others regarded them only as secondary invaders of a uterus which has suffered change as a result of endocrine disturbance. The latter view is accepted now as correct by the majority of workers and is in line with observations in other species. That the hormonal status of the animal is important in determining whether bacterial invasion of the uterus can occur is indicated by Rowson and Lamming (1954). They showed that in cattle the uterus is more susceptible to infection during metœstrus than it is in the œstral phase. Pyometra has been produced experimentally in the pig, guinea-pig and rabbit following the administration of œstrogens and in rabbits and guinea-pigs by fragmentation of the ovaries. Lesions of the anterior pituitary gland are said to predispose to the development of pyometra in women and perhaps this factor may apply also to the bitch.

A limited amount of work has been done on the role of hormones as a cause of pyometra using the dog as the experimental animal. Teunisson (1952)

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reports the following results. Of twenty experimental bitches, eleven developed pyometritis after a long course of œstrogen followed by progesterone. Seven of these eleven bitches had been ovariectomised previously. In six of the eleven bitches staphylococci were isolated from the uterine exudate. If the progesterone injections were withdrawn the symptoms disappeared but they recurred again when progesterone administration was resumed. If the bitches were not sensitised previously by œstrogen, it was necessary to treble the dose of progesterone to achieve the same result.

Pathogenesis

Fundamentally, pyometra is an endocrine disturbance in which the ovary plays an essential part. De Vita (1952) and Lesbouyries and Berthelon (1935) have observed that an oophorectomised animal will not develop pyometra. De Vita (1952), attempted, but failed, to reproduce the condition by giving a long course of œstradiol benzoate, using ovariectomised bitches. He recorded a bacterial invasion of the uteri but there were no characteristic changes in the endometrium. Oophorectomy of a clinical case resulted in recovery in five to fifteen days.

Hetzel (1935) suggested that the normal œstrus cycle of the bitch was disturbed by non-mating or non-conception with consequent proliferation and secretion of the endometrium. He produced a degree of glandular hyperplasia using œstrone.

Erichsen (1952) considered that the condition was fundamentally a hyperœstrogenism. He found that aged bitches had a tendency to endocrine disturbances and to inhibition of ovulation. If the inhibition was complete the result was a multi-cystic ovary. If the inhibition was partial, a long heat resulted with delayed ovulation—all the follicles present might not rupture and then both cystic follicles and fresh corpora lutea were found in the ovary. There might be a long heat followed by ovulation of all the existing follicles which would result in the ovary containing corpora lutea only. The result in any case was a hyperœstrogenism. With the protracted condition of hyperœstrogenism there are few, if any, leucocytes in the vagina, which might account for the high percentage of uterine infection in pyometra (the route of infection being via the patent cervix). Again, an excessive secretion of progesterone might inhibit posterior pituitary action and thus reduce uterine contractions, resulting in an accumulation of glandular secretion and regressional products in the uterus.

History

The majority of cases of pyometra occur in dogs ranging between six and nine years although a case has been recorded in a one-year-old bitch (De Vita, 1939). The oldest subject De Vita examined was fourteen years old. Cases occur more commonly in the nulliparous or uniparous animal than in the regular breeder. In many instances there is a history of functional disturbances of the reproductive system, i.e., abnormally long heat, phantom pregnancy, silent heat or undue frequency of the œstrus cycle.

Following such functional irregularities, some three to nine weeks after

œstrus, the bitch goes off her food and appears to be dull and listless with an increased thirst. She is depressed and dehydrated, the coat is rough and strong, and some bitches vomit occasionally. A variable proportion of cases show a vaginal discharge. If there is no vaginal discharge there may be some abdominal distension. In other cases there is an intermittent vaginal discharge which suggests a periodical opening and closing of the cervix.

Examination and Diagnosis

Several workers have pointed out that temperature records in cases of pyometra are of little prognostic value, due to the fact that thermal reaction is extremely variable, but a subnormal temperature is of significance and merits a guarded prognosis.

An examination of the vaginal discharge is of assistance in a diagnosis. It varies considerably in nature from being mucoid, slightly mobile and cream in colour to being thin, mobile, of a greenish-chocolate colour and containing small clots of blood. Some discharges are fœtid.

In the absence of a vaginal discharge, one may attempt to palpate the uterus, which has a doughy consistency. (In order to be certain of identifying the uterus it is necessary to locate the cornual bifurcation.)

Additional Aids to Diagnosis

The examination of a blood sample is of value for there is a marked leucocytosis. The white cell count varies enormously but a mean range would be between 20,000 and 80,000 white cells per cubic millimetre.

The differential count shows an increase in the number of immature neutrophils. There is an increase in the ratio of immature cells (stabs) to mature cells (segmenters), from 1 : 10 to 1 : 1.5. This is accompanied by a relative decrease in the number of lymphocytes. Haigler and Hawkins (1940) have described the presence of a characteristic neutrophil in cases of pyometra: a large cell with a smooth, rounded nucleus and many "toxic" granules.

There is an associated bone marrow hyperplasia.

It is generally agreed that there is no change in the number or nature of the erythrocytes. Rehfeld (1954) regarded the sedimentation rate to be of prognostic value—if the sedimentation rate was accelerated the prognosis was considered to be grave.

Various organisms have been recorded in the uterus of affected bitches including *Bacterium coli,* streptococci, staphylococci, Proteus and *Bact. ærogenes* but as the organisms are regarded as secondary invaders, probably entering the hyperplastic uterus during œstrus, they are of no special clinical significance.

Since it is not an uncommon experience to lose patients post-operatively as a result of a co-existent chronic interstitial nephritis, a means of differential diagnosis is essential. Examination of the urine tends to be misleading because the vaginal discharges present can lead to positive results in the protein tests. An estimate of the blood urea nitrogen is more reliable.

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Radiography may confirm the diagnosis. Lateral views are best but it is advisable to clear the colon and rectum by enema before the picture is taken.

Treatment

Various treatments have been recommended for pyometra including uterine irrigation (Stephenson, 1930), stilbœstrol and posterior pituitary extract (Brancker, 1943), diathermy (Khuen, *et al.*, 1940), oophorectomy (Lesbouyries and Berthelon, 1935), and ovaro-hysterectomy, but it is generally agreed that the latter is at present by far the most satisfactory method of treatment.

On those occasions when the immediate surgical risk is great it is of advantage to administer Lutenising Hormone. It is our practice to give 100 I.U. every second day and on four occasions. This line of therapy is supported by the observations and theories of Erichsen. The object is to attempt to improve the condition of the uterus and of the patient. In some cases the improvement is marked but it should be remembered that even if the condition be resolved there will probably be a recurrence after the next heat.

At operation, the uterus varies in its appearance. In a closed case it is distended, the uterine wall being thin and fragile. Engorged blood vessels will be seen running across the surface of the uterus, which is increased in length as well as girth. The increase in length may necessitate folding to accommodate the organ within the abdomen. Another feature in the closed type is the marked constriction of the uterus at the cervix. The closed case is typical of the pyometra of the metœstral phase.

The open case presents a different picture—the uterus is again a somewhat elongated organ, thicker than normal but not distended to the extent seen in a closed case. The bulk of the increase in the width of the uterus is represented by the increased thickness of the wall.

In the closed case the endometrium is reddened and may show areas of ulceration. These ulcers may be deep and cases have been reported (De Vita, 1939) where the ulcers have perforated the uterine wall leading to peritonitis and death. Cysts may be evident on the surface of the endometrium and serosa; these may be numerous.

The open cases may or may not show reddening of the endometrium. The endometrium may be grey and thrown up into ridges and folds; cysts may be found.

Histopathology

The Uterus. The surface ephithelial cells of the mucosa are enlarged and foamy in their appearance. In some cases they show a pseudostratification. Other cases show ulceration and loss of mucosa.

The principal changes occur in the lamina propria where the tubular glands show a marked hyperplasia. This hyperplasia may be so pronounced that the gland tubules may invade the annular myometrium. The deeper coiled parts of the glands often show a nuclear proliferation. As a result of the hyperplasia, the neck of the gland often becomes impacted with cell debris and exudate and thus the secretions are retained. This results in the development of retention

cysts in the lamina propria. In some cases the cystic changes are the predominant feature of the tissue changes. The cells lining these cysts are flattened and elongated with a dark staining nucleus. The cyst is surrounded by a variable amount of fibrous tissue.

Other changes are seen in the lamina propria. There is a marked proliferation of capillaries, particularly in open cases, and hæmorrhages into the tissues may be observed. There is a variable degree of cellular infiltration into the lamina propria. In the majority of cases the infiltration is predominantly by cells, which are mononuclear in type.

The myometrium in the bitch may be sub-divided into three zones. The innermost layer consists if a thick annular band of muscle which is surrounded by the stratum vasculare which consists of arteries, veins and nerves supported by connective tissue. Surrounding these two layers is a thin longitudinal layer of muscle. In a proportion of cases the deeper tubular glands may invade the annular myometrium accompanied by a cellular infiltration from the lamina propria. The cells are seen infiltrating between the muscle fibres. The infiltration may extend throughout the annular myometrium and into the stratum vasculare. It is not unusual to find a phlebitis. The whole uterus is surrounded by a serosa which is a direct continuation of the broad ligament and is closely associated with the longitudinal myometrium.

The Vagina. Examination of the vaginal mucosa has shown that the changes may be of a metœstral type, namely, the stratified squamous epithelium of œstrus is lost to be replaced by a columnar epithelium. There may be a few leucocytes infiltrating through the mucous membrane. A high proportion of cases show a pro-œstral/œstral state of the vaginal mucous membrane, that is, a thick stratified squamous epithelium, although pyometra occurs in the metœstral phase of the cycle. According to Bloom (1954) the majority of cases of pyometra show a vagina in the pro-œstral/œstral phase but there are certain morphological variations. The stratum corneum may be absent or the superficial squamous cells are "ballooned." They show a moderate infiltration with mononuclears and the usual pro-œstral œdema is absent.

The Ovary. The ovary practically always contains corpora lutea, a high proportion of which are degenerate. It is not uncommon to find ovarian cysts in cases of pyometra which may or may not be associated with corpora lutea. In the closed cases the corpora lutea are generally fresh but in the open cases the majority show regressive changes. In some cases the ovary is reduced to a multi-cystic mass but even under these circumstances the vestiges of ovarian tissue may show oogenesis.

De Vita (1939) recorded a percentage of cases of ovarian tumour associated with pyometra—these were probably granulosa cell tumours. Granulosa cell tumours are often accompanied by pyometra but pyometra is not often due to granulosa cell tumours.

Other lesions. A number of cases may show secondary abscesses in liver, spleen and kidney associated with a uterine phlebitis. Hypertrophic skin changes and alopecia have been observed.

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Prognosis

Tufresson (1953) studied the prognosis of pyometra after ovarohysterectomy. Of 273 cases, 44 died during the operation or whilst in hospital. On the "follow-up" of 209 cases, 163 were completely normal. Thirty-seven died of unrelated conditions and nine cases died as a result of unfortunate sequelæ as recorded below:—

Posterior paralysis, 3; incontinence, 3; hernia, 2; bloody discharge, 1.

From these results it is evident that the prognosis in pyometra is good and that ovarohysterectomy remains the treatment of choice, at least, until the pathogenesis becomes clearer.

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THE POST-NATAL DEVELOPMENT OF THE HORN TUBULES AND FIBRES (INTERTUBULAR HORN) IN THE HORNS OF SHEEP

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THE development and growth of the horn in the sheep does not appear to have been studied in detail before. Passing reference has been made to it in numerous publications devoted to similar studies in the ox and other animals.

Youatt (1869) stated that the vascular membrane between the bone core and the horn covering has the power of forming, at the same time, two altogether different structures; its inner surface forms the bone which is added to the external surface of the bony axis, while the outer surface adds new layers of horny substance on the internal surface of the horny sheath,, i.e., that this vascular membrane presents the dermis on the one hand and the malpighian layer of the epidermis on the other. Fambach (1909) mentioned that the young horn consists only of a thick epidermis, the cell layers of which are difficult to differentiate and lie stacked parallel to the surface of the matrix. With the development of the permanent horn the papillæ of the cutis begin to form horn cylinders at the apex and continue towards the base and sides. The fully-formed horn is differentiated into a suprapapillary layer which is secreted from the

papillæ, and the interpapillary horn layers which are secreted from their intervening layers. The suprapapillary horn consists of open cylinders which are held together by the horn substance. The firmness of the horn depends on the cornification of the suprapapillary cylinders. Helmut (1942) was of the idea that keratinisation runs lamellary, single lamellæ are bag-shaped and their course is not straight but bulges like arcades, which give the appearance of grooves and elevations of the horn surface. Trautmann and Fiebiger (1952) stated that the main substance of the horn is composed of fine tubules, which contain medullary substance in the central portion of the horn only, otherwise they contain air. The horn tubules are connected by loose intertubular horn, which is very abundant in sheep.

Present Study

The microscopic study of the development of the horn covering was carried out on a series of sections taken from the horn tissue in varying stages of formation; forty fresh sheep's heads of different ages ranging between a few hours after birth and five years were used. The specimens were prepared for sectioning by two methods: the paraffin and the freezing methods. The hard, horny tissue was softened by two methods. The first was to macerate the tissue in 2 to 5 per cent sodium hydroxide and the second by using wetting agents (detergents). Hæmatoxylin and eosin, and phloxine tartrazine stains were used to demonstrate the horn fibres and tubules.

During the first week after birth in male lambs, the tissue structures in the horn site do not show any considerable development beyond that of the last few days of the gestation period. The epidermis still consists of four strata which are in succession from inner to outer, germinativum, spinosum, granulosum and corneum. The stratum corneum consists almost entirely of horn tubules and fibres (intertubular horn). They lie alongside each other and alternate with each other (Fig. 1). The cells at the bottom of the horn tubules still show the granular cells of the stratum granulosum (Fig. 2). These cells have lost their cytoplasm and most of their granules, and their walls are ill-defined. As these cells are traced upwards, they appear swollen, irregularly shaped and frequently lacking their nuclei. The cells appear either vacuolated or containing a homogeneous substance. A few granules can still be seen in the upper cell layers of the horn tubules. The horn fibres have granular cells, roughly shaped in the form of a flattened diamond (Fig. 3). The granules have an affinity for hæmatoxylin but their response to the stain is somewhat variable and the various degrees of colour indicate the degree of keratinisation of the granular cytoplasm. The granules darkly stained with hæmatoxylin are still in the keratohyalin stage, while those faintly stained with hæmatoxylin are beginning to show the pink colour of the eosin stain and denote the beginning of keratinisation in these cells.

Microscopic examination of the horn sections of lambs of two to four weeks of age portray a considerable degree of development. In the male lambs the horn tubules show two stages of development (Fig. 4). The first, found towards the free surface, where the tubules appear to contain degenerating cells that have broken down leaving only a few keratinised bridges crossing the tubules transversely and connecting the two horn fibres situated on either side of each

tubule. These bridges were once the cell walls of the degenerating granular cells. The horn tubules appear to contain a homogeneous substance which, in the opinion of the writer, sticks the horn fibres together. The other stage of development of the horn tubules is the primary stage of formation which is seen during the first week. These newly-formed tubules lie close to the stratum granulosum. They consist of broken-down granular cells with ill-defined cell walls in their deeper parts and irregularly-shaped smaller cells in the superficial parts.

The horn fibres (intertubular horn) also present two stages of development (Fig. 4). The first is in the superficial or previously formed fibres which now appear mostly keratinised and readily take up the eosin stain. They are scale-like cells with an undefined outline. The other stage of the fibres is the primary one which was described previously. In this the fusiform granular cells are closely arranged with the long axis of each cell directed longitudinally. They still retain the keratohyalin granules which take the hæmatoxylin stain. The most superficial regions of the stratum corneum begin to have a wavy appearance. This may be due to the difference in length of the horn fibres or to the different degree of development of each fibre. The horn fibres and tubules of the sides and surfaces of the horn are all directed towards the apex.

In male lambs of 4 to 6 weeks of age, the outermost layers of the stratum corneum, which are older than the deeper ones, begin to present the appropriate structure of the mature horny covering. It consists of wavy, horny fibres closely packed together in small bundles. These bundles are separated from each other by the horn tubules which appear as empty spaces crossed by horny bridges connecting the two bundles on either side of a horn tubule. There are still a few compressed and elongated nuclei which can be seen here and there in the stratum corneum. The deeper layers of the stratum corneum still present the horn tubules and fibres at an early stage of formation similar to what have been described during the previous stages.

In female lambs of 4 to 6 weeks, the first signs of horn tubules and fibres (intertubular horn) begin to be formed in the stratum corneum. The way of their formation is similar to that of the male. At this age, clusters of degenerating cell masses of the stratum granulosum begin to extend in the form of columns through the stratum corneum and form the early horn tubules. The horn tubules and fibres continue to develop as the lamb increases in age and on reaching 6 to 8 weeks, the stratum corneum at the horn site appears to consist almost entirely of horn tubules and fibres. They lie alongside each other and alternate with each other. The horn tubules consist of granular cells, which once constituted the stratum granulosum, becoming distended and irregularly shaped. Large vacuoles are seen in their cytoplasm, and their nuclei are still recognisable. The horn fibres consist of fusiform granular cells, closely packed together and with the long axis of each cell attain a more or less vertical rather than a horizontal direction.

On reaching the age of 8 to 12 weeks, the microscopic picture of the horn tubules and fibres is more or less the same in both sexes. It is important to note

PLATE I

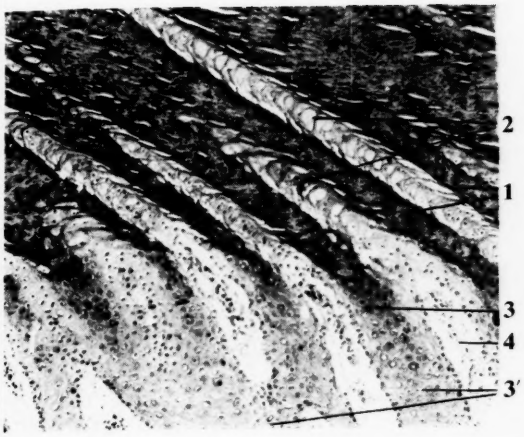

Fig. 1
1st—2nd week stage. Male lamb.
Epidermis at the horn site. x 120. (1) horn fibre, (2) horn tubule, (3) stratum granulosum, (3') stratum spinosum, (4) dermal papillæ.

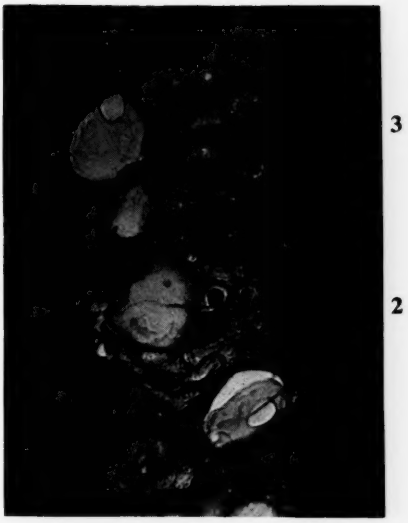

Fig. 2
1st—2nd week stage. Male lamb.
Horn tubule. x 450. (1) horn fibre, (2) cells at an advanced stage of degeneration (distended without their granules), (3) cells at an early stage of degeneration (slightly swollen and still retaining their nuclei and granules).

Fig. 3
1st—2nd week stage. Male Lamb.
Horn fibre. x 620. (1) horn fibre, (2) horn tubule.

(*Article by George, page 30*)

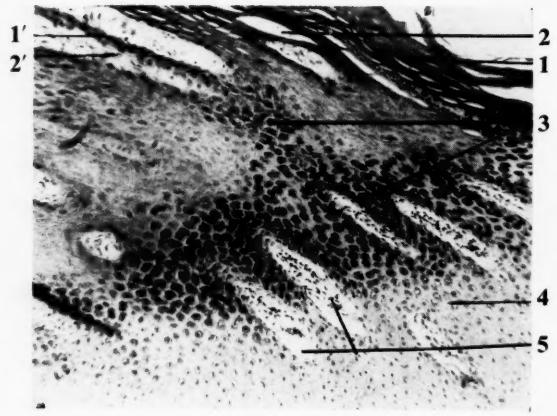

Fig. 4
2nd—4th week stage. Male lamb.
Epidermis of the skin at the horn site. x 120. (1) advanced stage of horn fibres, (1') early stage of horn fibres, (2) advanced stage of horn tubule, (2') early stage of horn tubule, (3) stratum granulosum, (4) stratum spinosum, (5) dermal papillæ.

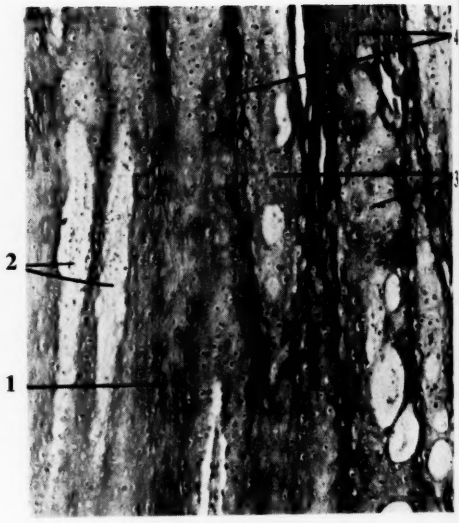

Fig. 5
8th—12th week stage. Male lamb.
Direct formation of stratum corneum from the stratum spinosum. x 115. (1) stratum spinosum, (2) dermal papillæ, (3) horn tubules, (4) horn fibres.

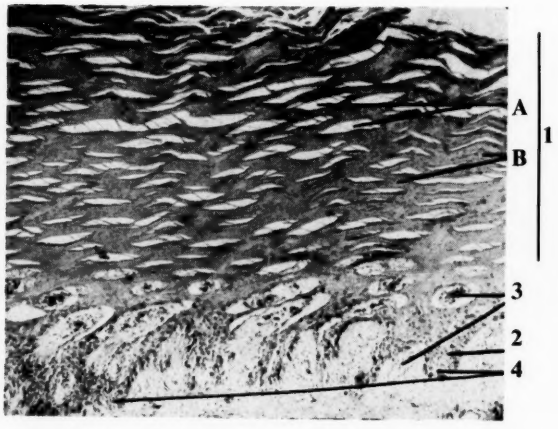

Fig. 6(*a*)
8th—12th week stage. Female lamb.
Epidermis and dermis of the horn of the female. x 85. (1) stratum corneum: A, horn tubules; B, horn fibres; (2) stratum spinosum; (3) dermal papillæ; (4) Inter-papillary pegs.

(*Article by George, page* 30)

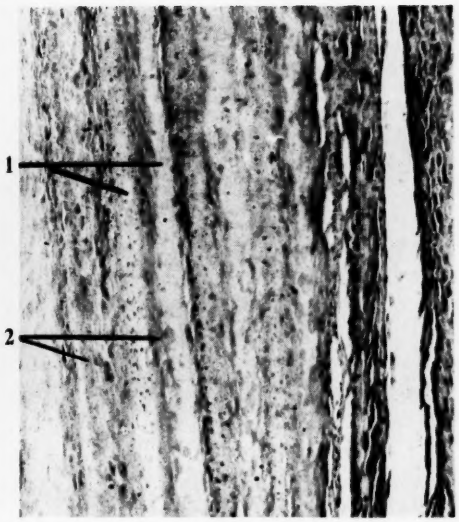

Fig. 6(*b*)
8th—12th week stage. Male lamb.
Horn fibres and tubules at an advanced stage of development. (1) horn tubules; (2) horn fibres.

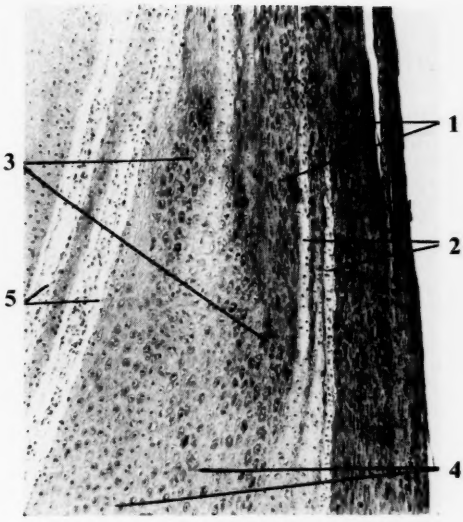

Fig. 7
8th—12th week stage. Male lamb.
Epidermis covering the base of the horn. x 110. (1) horn fibres; (2) horn tubules; (3) stratum granulosum; (4) stratum spinosum; (5) dermal papillæ.

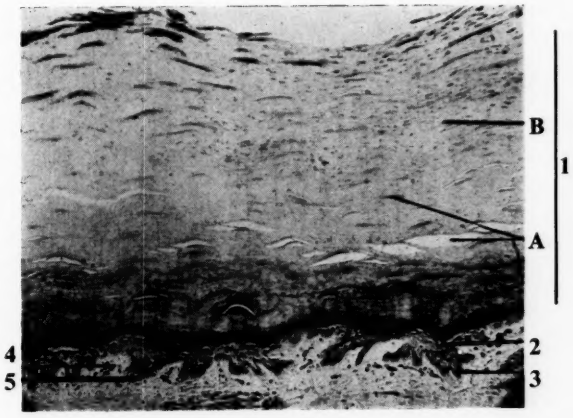

Fig. 8
52 week stage. Female lamb
Epidermis at the base of the horn. x 110. (1) stratum corneum: A, horn tubules; B, horn fibres; (2) stratum spinosum; (3) stratum germinativum; (4) dermal papillæ; (5) interpapillary pegs.

(*Article by George, page* 30)

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that from this age the cells of the stratum granulosum show two different ways of changing into the stratum corneum. Those located at the base of the horn behave in the usual way forming keratinised cells, i.e., the cells of the stratum spinosum change into the granular cells of the stratum granulosum and these in turn change gradually into keratinised cells forming the stratum corneum. In the other method of keratinisation the cells of the stratum granulosum are immediately keratinised, forming the stratum corneum without the formation of keratohyalin granules (Fig. 5). This happens in the epidermis covering the apex of the os cornu. Thus, one can recognise the cells of the stratum spinosum almost immediately followed by the keratinised cell layers of the stratum corneum, there being little or no sign of the stratum granulosum. This phase of keratin formation without passing through the keratohyalin stage was also recognised in the horns of ruminants and hooves of solipeds and ruminants as stated by Trautmann and Fiebiger (1952). They also stated that the change of the stratum spinosum into stratum corneum without the formation of keratohyalin is characteristic of hard horn.

Moreover, Gadow (1902) and Fambach (1909) mentioned that there are young horn and permanent horn. The young horny tissue is formed in the early days of the life of the animal and it falls off gradually as the animal advances in age, and becomes replaced by the permanent or hard horny tissue. This occurs gradually, taking a few months. As a result of this present work, after examining the serial sections of the horn of this stage it has been found possible to agree with the findings of the above-mentioned workers. The stratum corneum at the base of the horn covering and the outer keratinised layers of the apex of the horn covering are soft or young keratin and it gradually falls off in the form of scales. The rest of the stratum corneum of the horn covering is hard or permanent horn.

The horny fibres (intertubular horn) of the hard and soft keratin differ from each other while the horn tubules are almost the same. The horn tubules consist of degenerating cells of the stratum granulosum and contain a homogeneous substance. Some of the tubules appear to be empty and only present a few keratinised bridges connecting the horn fibres on either side of the horn tubule (Fig. 6*a*). In soft keratin the horn fibres consist of granular cells at different stages of keratinisation. This can be demonstrated by hæmatoxylin and eosin stain as mentioned for the previous stage. The cells are arranged close to each other with their long axis placed vertically, or in other words, parallel to the long axis of the horn (Fig. 7). The horn fibres of hard horn (Fig. 6*b*) connsist of cells with ill-defined cell walls. The cells which are close to the horn tubules appear to be flattened, empty, and with keratinised cell walls.

At the age of 16 weeks the horns of both sexes attain the appropriate shape and structure of adult horns (Fig. 8). At the base of the horn the stratum corneum still shows the granular cells in its horn fibres (intertubular horn) and the degenerating cells are still present in some of the horn tubules. In the central part of the horn the stratum corneum has most of the horn tubules empty, but some of them contain a homogeneous substance. This agrees with the findings

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of Trautmann and Fiebiger (1952): they state that the horn tubules, placed in the central part of the horn, contain medullary substance, but otherwise they contain air. The stratum corneum of the horn covering beyond the apex of the os cornu is highly developed. The horn tubules are mostly empty, with a few transverse horny bridges connecting the horn fibres on either side. There are still some nuclei of the degenerating cells which once filled the tubules. These can be seen, here and there, in some of the horn tubules. These nuclei are usually accompanied by a homogeneous substance which probably has some connection with the cementing of the fibres together. The horn fibres (intertubular horn) consist of masses of dead cells firmly welded together by keratin. Some of the cell outlines may still be vaguely seen. Pigment granules can also be seen embedded in the keratinised mass.

Acknowledgments

I wish to express my thanks to Professor T. Grahame, in whose department this work was carried out, for his advice and encouragement. My thanks are also due to Mr. E. Roberts for his technical advice, to Mr. R. Hood for the photographs, and to Animal Disease Research Association for making available such material as I required.

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STUDIES ON CONTAGIOUS BOVINE PLEUROPNEUMONIA

With Special Reference to the Complement-Fixation Test

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PART I

Introduction

IN Nigeria, the Complement-Fixation Test for Contagious Bovine Pleuropneumonia was first used for diagnosing the disease from serum samples sent in from the field, but it had its limitations because of the rapidity with which acute cases can develop and negative reactors become positive, and to the slowness of communications between the field and the laboratory. However, it was thought that the test might prove a valuable aid to laboratory studies on immunity to this disease, and serological records have therefore been kept of all the animals used in experimental work with pleuropneumonia vaccines.

The objects of this work on pleuropneumonia are:—

(*a*) a safe, simple and efficient means of causing a lasting immunity.

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(*b*) a simple and reliable test for detecting the disease in inapparent cases.

(*c*) a satisfactory method of challenging immunity, for comparing the efficiency of vaccines.

Methods of Immunisation.

(i) Primitive methods practised by native cattle-owners. These consist of the introduction of virulent material under the skin, usually over the nasal bones. Such methods, while not to be recommended—on humanitarian grounds, if for no other reason—are reputed to be effective as regards life-long immunity, but are dangerous, especially in inexpert hands. The method as practised by the Fulani in Nigeria (Coulthard) consists of the introduction of diseased lung tissue, ground up in milk and usually mixed with certain herbs, under a flap of skin over the nasal bones. As soon as swelling commences, the area is ringed round in a double circle with a red-hot iron. The inner circle of the cauterised area is then cut out with a knife. Any hæmorrhages are recauterised, and the wound dressed with an astringent powder. The lesion usually heals fairly quickly with no untoward sequelæ other than disfigurement, and immunity is said to be life-long. Mares (1951) has described similar methods in Somaliland.

(ii) The Willemsian method. Willems (1852) advocated the injection of fresh virulent pleural exudate into the tip of the tail as a means of immunisation. This was usually followed by swelling of the last few inches, with the not infrequent loss of part or all of the tail by sloughing. Sometimes the tail reaction spread sufficiently to involve the gluteal muscles, and this usually resulted in the death of the animal. Modifications subsequently introduced include the addition of glycerine to the fluid used for inoculation, to kill some of the contaminant bacteria which were considered responsible for especially severe reactions, and firing a ring round the tail above the commencing reaction to control its spread. Virulent cultures can be used instead of pleural exudates. Severe reactions can be obtained after the use of pure cultures, showing that it is not only contaminants that are responsible for accidents, although they doubtless aggravate the condition. This method is wholly unacceptable to Nigerian cattle-owners, who, while tolerating the disfigurement of the face caused by the previous method, consider that a cow without a tail, or even with half a tail, is not a cow at all. Immunity is, however, believed to be life-long.

(iii) Vaccination with attenuated cultures. Various workers (Walker, 1921; Knowles, 1927; Bennett, 1932; Campbell, 1938*c*; Purchase, 1939; Hobday, 1945; and many others) have found that, after a number of generations, sub-cultures of the organism lose their pathogenicity before their power of immunising. Campbell found a direct correlation between the virulence of a strain and its immunising power, although Knowles claimed that there was none. There is certainly considerable variation between one strain and another, and it is not possible to say—as some of the earlier workers tried to do—at which generation cultures can be used without danger, and at which generation they cease to immunise. This can only be ascertained by experiment with the particular strain in question. It has been customary to inject the less attenuated strains into the tip of the tail, where the risk of severe reactions is less than in the neck or behind

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the shoulder. Alternatively, immunisation may be attempted with two or even three strains of progressively decreasing degrees of attenuation at intervals of from one to three weeks. Under Nigerian conditions, tail inoculation is not desirable, as half-trained assistants frequently find difficulty in getting the inoculum into the tough and scanty subcutaneous fascia of the tail, and the less-conscientious among them may even deposit the dose between the hairs of the tail-tuft.

(iv) Vaccination with egg-adapted strains. In Kenya, promising results have been obtained (Sheriff and Piercy, 1952, 1953) with strains of the pleuro-pneumonia organism adapted to the chorioallantoic membrane of developing hens' eggs. No information is yet available as to how long the resultant immunity lasts.

Diagnostic Methods.

Clinical diagnosis of pleuropneumonia can present many difficulties, owing to the number of atypical cases, subclinical infections, and apparently recovered animals which still harbour infective sequestra in their lungs. Search has therefore been made from the earliest days of bacteriology onwards for a satisfactory test.

(i) Allergic tests. Arloing (1895) described allergic tests with " pneumo-bacillin " which were later discredited, especially when his *Pneumobacillus* was shown not to be the primary etiological agent of the disease at all. No satisfactory allergic test appears to have been described.

(ii) Precipitin tests. Ono (1925), giving virulent cultures to rabbits, found that intra-ocular injection caused an iritis and intra-testicular injection a non-suppurative swelling of the testicles. The serum of the recovered rabbits then gave a precipitin reaction with serum from infected cattle.

(iii) Complement-fixation tests, both hæmolytic and conglutinating, have been tried by various workers (Heslop, 1921, 1922, in Australia; Walker, 1923, in Africa; and many others in Central Europe and Japan. Most of these found difficulties of one sort or another, and the first really satisfactory method was that of Campbell and Turner (1936), which set a standard for all time. Improved antigens were introduced (Campbell, 1938*b*; Campbell and Turner, 1953), but otherwise very little difference seems to have been made in the actual technique of the test. This test has been carried out in Nigeria, with a few very minor modifications, and has proved completely reliable. It is, however, complex and requires laboratory facilities. If a simpler form of test, equally reliable, could be found, this would be a great advantage, but the C.F. test would probably still be required for checking doubtful cases.

(iv) Agglutination tests. Many of those working on C.F. tests have at the same time tried agglutination tests, and in the early days some found these more satisfactory than the C.F. techniques available at the time. Campbell (1938*b*) found the agglutination test less sensitive than the C.F. test. These tests were mostly tube tests. Priestley (1951, 1952), in the Sudan, described a rapid slide agglutination test which he considered more sensitive than the C.F. test. Newing and Field (1953), in Nigeria, have described a rapid slide test using a stained antigen and whole blood, which promises to be most useful as a field test.

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Methods of Challenge.

The difficulty of setting up this disease artificially has always been a great handicap to laboratory studies. Chauveau and Nocard (quoted by Nocard and Leclainche, 1903) set up one case of the disease by connecting the head of a healthy animal to that of an infected one by means of a linen tube, but simpler forms of contact between healthy and infected animals are apt to give very uncertain results. The following methods have been studied by various workers with a view to challenging immunity.

(i) Subcutaneous inoculation. Virulent material inoculated subcutaneously produces a highly-characteristic local lesion, but one which bears little general resemblance—apart from certain histological details—to the naturally-occurring lesions in the lungs. A swelling appears, usually during the second or third week, commencing as a soft, subcutaneous œdema but soon becoming firm and tense. It may take the form of a prominent lump, but it is more commonly flat. It slowly extends until it may be 12 inches or more across. Large swellings are in most cases fatal.

(ii) Intravenous inoculation. Provided that no material leaks out into the perivascular fascia or subcutaneous tissues to set up lesions of the type referred to above, cultures or uncontaminated exudates can usually be inoculated intravenously without causing lesions of any sort. Nocard and Roux (1903) inoculated material into an ear vein and then cut off part of the ear to avoid any risk of local reaction. There was no reaction of any sort to the inoculation and no immunity resulted. However, Daubney (1935) in Kenya and later Mettam and Ford (1939) in Nigeria succeeded in setting up more or less typical lung lesions by the intrajugular injection of plugs of agar containing the organism. These plugs set up embolisms in the pulmonary vessels in which the organism was localised and able to multiply and spread through the infarcted lung tissues. Longley (1951) was able to set up lung lesions of the closely related caprine pleuropneumonia in goats by damaging the intima of the smaller pulmonary vessels by the intravenous injection of chloroform, which led to thrombosis and multiple infarcts; 24 hours later he injected cultures of the caprine organism which was held up in these infarcts and was able to establish itself. A small test in Nigeria to repeat this procedure in cattle was unsuccessful, but it is worth further investigation. Hobday (1945) found that it was sometimes possible to get lung lesions after intravenous inoculation if he made a number of punctures into the lungs with a long, sterile needle, as this gave an opportunity for the circulating organisms to settle down in the damaged tissues and multiply.

(iii) Intratracheal inoculation. Daubney (1935) produced the disease in one case out of twenty by the intratracheal inoculation of filtered exudate. Campbell (1938*a*) successfully produced typical cases in all of six animals by introducing virulent cultures into the terminal bronchioles by means of a fine catheter passed down the trachea. Mettam and Ford (1940) produced two typical cases in three animals by the intratracheal injection of agar plugs containing the organism, and decided that this method was superior to all others, as they obtained complete involvement of one lung instead of the numerous small and rapidly sequestrating lesions they had found after giving agar plugs intrajugularly.

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(iv) Atomised spray. Since it is believed that in the field the disease is spread by infected droplets in the exhaled breath of infected animals, especially when these are coughing, Australian workers (Campbell, 1938*a*) have tried spraying virulent cultures in the form of a fine mist into a small closed stable where the animals were confined. Results have been so excellent that this is now their standard method of challenging experimental animals. Similar methods have been used with success in Rhodesia (Hobday, 1945, 1949). Some form of lung damage, either mechanical or due to the presence of helminth parasites, appears to assist the setting-up of infection in this way.

All these methods, however, involve certain technical difficulties, and workers in various parts of the world have recently tended to fall back on the old method of subcutaneous inoculation, in spite of the disadvantage that this causes a very different type of lesion from that against which the vaccine is is intended to produce an immunity—Priestley (1953) in the Sudan, and Sheriff and Piercy (1952, 1953) in Kenya. In Nigeria, this technique has been modified by considering the serological reaction to the challenge material (dried pleural exudate) as well as the local lesion produced, if any, and this makes the ordinary subcutaneous challenge a much more delicate test. The method, and the observations which led to its adoption, are described in the following report on experimental work in Nigeria.

Experimental Work

A. Observations made using the Complement Fixation Test.

(i) *Technique and method of recording results.* The test as carried out in Nigeria is to all intents and purposes that of Campbell and Turner (1936) and the antigen used has been very kindly supplied by Dr. Turner himself. The actual details of the technique, however, have been adapted from Hole and Coombs (1947). As this has been published in full, nothing but the briefest summary is required here, and only the points in which the latter authors' technique for C.F. tests in general differs from the classical Campbell and Turner test for pleuropneumonia will be mentioned. The sheep's corpuscles are used as a one per cent suspension instead of three per cent. The serum is diluted in double series so that a control tube can be provided for each dilution. The serum-complement-antigen mixture is left to react at room temperature instead of in the water-bath. The water-bath is, however, used after adding the hæmolytic system. It has not been found necessary to shake the tubes during the reaction. Before the results are read, the tubes are centrifuged at 2,000 to 3,000 r.p,m, for three minutes. The cells, if any, are deposited and the degree of hæmolysis is indicated by the colour of the supernatant fluid or, where this is almost complete, by the amount of cells deposited. The end point should, strictly speaking, be the last tube where fixation is complete (i.e., the supernatant fluid water-clear), but in actual practice the titre is taken as the last dilution to give at least 75 per cent fixation (i.e., not more than 25 per cent hæmolysis). This is done because some sera, owing to the presence of inhibitory substances, never show complete fixation although otherwise of moderately high titre. Similarly, where a number of consecutive tubes show partial fixation

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(c.50 per cent) the tube previous to the last of these is taken as the end point. Twenty-five per cent fixation is referred to as a trace, and smaller amounts as a slight trace. These four degrees of fixation, read by subtracting the percentage hæmolysis from 100, correspond roughly to Campbell and Turner's categories 4, 3, 2 and 1 respectively. Traces or partial fixation are only referred to where they occur at 1 in 10 which is the first dilution tested. The optimal antigen dilution is found by experiment, and is the dilution which gives the highest titre. Stronger as well as weaker dilutions will give a slightly lower titre than is obtained with the optimal dilution.

Fig. 1. Titres of three animals vaccinated with an early subculture of Sudan strain F 48 in varying doses.

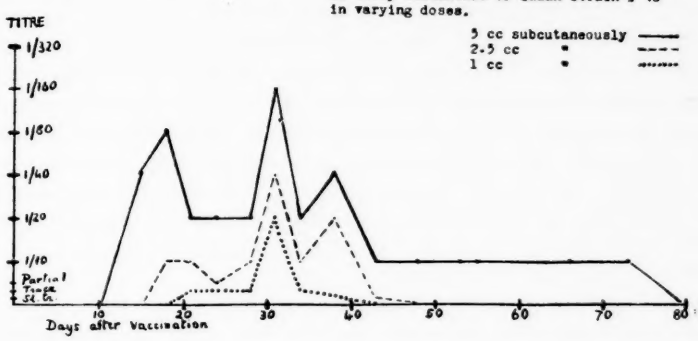
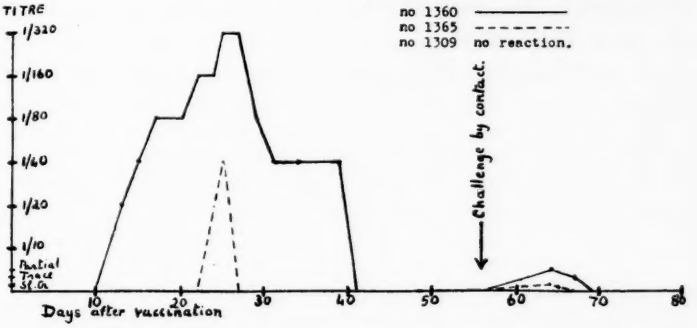


Fig. 2. Reactions of three animals vaccinated s/c with 1cc of KHJ/J, and later challenged by close contact with sick animals



Positive and negative sera should be included with all batches tested. In this laboratory a standard positive control of known titre (1 in 80) is used. This is collected in bulk and freeze-dried in 2 c.c. ampoules which are stored in the refrigerator. Before one batch is exhausted another suitable sample is collected, standardised against the original, and kept to replace it later. From time to time apparent fluctuations in the titre of this standard are observed. While it is conceivable that the titre might fall slightly after long storage, it is most unlikely that it could rise. Yet the variations observed have usually

indicated a rise in titre. An exact explanation of this has not been found, but it is believed to be due to variation in the quality of the sheep's cells used. Accordingly, when the daily titration of the complement is being made, this is not always interpreted strictly in accordance with classical procedure, but allowances are made and the dose of complement used in the test is that which will give the correct titre with the standard serum.

The terms "fleeting" and "transient" reactions which are often used in the literature should be carefully distinguished. A "fleeting" reaction is one where the antigen-antibody complex is bound only lightly to the complement. At first the serum appears positive, but after a short while the complement is liberated again and lysis of the sensitised cells takes place. Fleeting reactions are associated with unsuitable types of antigen. They were a great source of trouble to the earlier workers, but should not be encountered with Campbell and Turner's antigen. "Transient" reactions are low titre reactions of short duration frequently seen when animals are introduced into an infected environment, and are believed by Campbell and Turner to result from abortive infections quickly thrown off. As animals which have shown transient reactions frequently become infected a fairly short time afterwards, these abortive infections do not necessarily produce any immunity, and may sometimes even sensitise the animal to subsequent infection. They occur in resistant animals as well as susceptible, when these are exposed to infection (see Fig. 2).

In Nigeria, a similar type of transient reaction has been noted from time to time in vaccinated or recovered animals, long after the titre has become negative, and without the animal being exposed to any infected environment. This is presumably due to some release into the circulation of antibody stored elsewhere. Animals which show reactions of this type should be avoided for use in experiment, as they may already have considerable resistance. This will be discussed more fully later.

(Part II will appear in February issue)

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REVIEWS

A Dictionary of Dairying, by J. G. Davis, D.Sc., Ph.D.(Lond.), F.R.I.C., M.I., Biol., F.R.San.I., 1955. Second Edition. London: Leonard Hill, Limited. Pp. 1132 + xxviii. Many figures and tables. Price 65s.

The first edition of this dictionary was published in 1950 and it speaks well for its success that a new one is called for within five years. The experiences of the author as a dairy bacteriologist and chemist allow him to appreciate the research, technical, legal and commercial aspects of milk. More than sixty contributors help him and certain sections of the book are the work of authors of repute in their own sphere. There is a list of the books which are referred to in the text and these number over seventy; this gives some indication of the breadth of this dictionary which covers the field from cows to the consumer.

Even the most experienced individual in the milk industry needs books of reference and this is one of the convenient kind. It is arranged in alphabetical order and will be valuable to dairy scientists, technologists, managers of dairy plant, dairy farmers and others connected with the milk industry. There are photographs and drawings, tables and references, as well as text; many of the articles are long and authoritative. For example, "Bacteriological grading of milk" is listed under "B" and is approximately 8,000 words in length; it has seven tables and about two dozen references. Many of the contributions are, of course, less in quantity, but they range over such subjects as "Mechanical handling in the dairy industry," "Pasteurisation," "Pasteurisation, alternatives to," "Vitamins," "Thermophilic bacteria," "Water supplies for dairy purposes," "Ropy milk," "Infant foods," "Cheese," "Ice cream," "Feeding of dairy cattle" and "Farm advisory work."

The comprehensive nature of the work and its catholic outlook impresses upon one the complicated nature of milk production and the many ramifications of the industry. The passage of milk from the dairy farm is long and varied before it reaches the consumer in some edible form. This progression impinges upon bacteriology, chemistry, physiology, farming, engineering, physics, public health, nutrition and animal health, economics and the law. A dictionary of dairying is the useful handbook for supplying the knowledge connected with these aspects of milk production.

Mechanisms of Microbial Pathogenicity: 5th Symposium of the Society for General Microbiology. Edited by J. W. Howie and A. J. O'Hea. London: Cambridge University Press. Pp. 333 + x. 25s. net.

The Society of General Microbiology have focused attention upon many of the problems of current interest to microbiologists by recording the results of symposia held under their guidance and care. Adaptation in micro-organisms, the nature of the bacterial surface, the nature of virus multiplication, and autotrophic bacteria have come within their scope. The present one is the fifth in

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the series and is probably of greater interest to pathologists and physicians than some of the earlier ones. It attempts to deal with the processes and mechanisms whereby microbial multiplication within a host may lead to disturbances of function or even the death of the host. It is not easy to divorce the host from a host-parasite relationship and to discuss pathogenicity without relating it to the host. In fact, it is not possible to separate the one from the other in such a relationship because pathogenicity can only be defined in terms relative to the host. It has been possible, however, to analyse the terms which are used, in writing of pathogenicity, virulence, resistance and susceptibility. This analysis demonstrates once again that the actual reactions of a host to an invading organism may, on occasion, contribute more to pathological changes than any inherent aggressive behaviour of the micro-organism. All micro-organisms are not dangerous to man and higher animals, and this truth is well illustrated in the suggestion that virulent mutants of an avirulent strain of bacteria may arise; these exercise their virulence because they have lost the power to stimulate the normal and primary defence mechanisms of the host. A loss occasioned by a micro-organism may therefore be detrimental to its host.

Sixteen contributions comprise the symposium which was held in London in April, 1955; the contributions range from those dealing with the general aspects of pathogenicity to those of a special nature. Consideration is given to the rôle of bacterial toxins as well as the ability to multiply in a host. Special contributions are concerned with some of the toxins formed by corynebacteria and by clostridia and one suggestion is made that it may be useful to look for feeble toxins in anthrax and pneumococcal diseases; these are examples in which a lethal effect is not produced until there has been considerable multiplication of organisms in the body of the host. It was left to other contributors to point out that so far as the pathogenicity of *B. anthracis* is concerned, there are three important substances all with aggressive activity. By studying the products of the growth of the organism *in vivo* it has been shown that two of these substances are intracellular whereas the third, which is connected with the lethal factor in anthrax, is produced extracellularly.

In his contribution to the studies on the pathogenicity of tubercle bacilli and the development of tuberculosis, Dubos suggests that virulence exists in many degrees and forms a continuous spectrum. The extent of the manifestations of disease depends upon the physico-chemical characteristics of the *in vivo* environment in which the infection process follows its course. In the infective granulomata, attention must be paid especially to the multiplication of the micro-organisms and this throws into relief the relationship between infection and such disturbances in the host as uncontrolled diabetes and of starvation in which ketosis is a feature.

The pathogenicity of protozoa depends primarily on whether they may establish themselves in a host; pathological effects may then arise when many parasites are killed and disentegration products are liberated and excite reactions of a mechanical or an allergic nature. Hosts which are to survive should learn to get rid of parasites without undue disturbance. Some protozoa may kill by

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intensity of infection and others by injury to a particular organ, and the comparative reactions in vertebrate and invertebrate hosts yield interesting data.

The mechanisms of pathogenicity exhibited by fungi which are parasitic on man and higher animals are diverse and complicated. It appears as if a fungus may be pathogenic on one occasion and saprophytic on another; a pathogen may sometimes be limited to certain tissues of the host and on another produce a generalised infection.

Further contributions on plant pathogens and their enzymes, on the toxins and bacteria in plant diseases and on the invasion of plants by vascular pathogens, complete the record of a symposium which cannot help but stimulate all who are interested in microbiology. The Society for General Microbiology was founded in order to weld together those who pursued their studies in different fields and along different "microbiological" paths. The fifth symposium adds to the evidence which shows how well the Society is achieving its objective and is a stimulating and instructive contribution to microbiology.

VETERINARY REVIEWS AND ANNOTATIONS

A NEW journal consisting of two issues per annual volume makes its appearance under the above name. It is prepared by the Commonwealth Bureau of Animal Health and published by the Commonwealth Agricultural Bureaux at 25s. per annum.

The first part of volume one is dated April, 1955, and consists of a long review by Professor R. Lovell on "Intestinal diseases of young calves with special reference to infection with *Bacterium coli*" and two shorter reviews; one on "Coccidial and other forms of parasitism in their relation to poultry husbandry" is by Dr. C. Horton-Smith and the other by Dr. Phyllis G. Croft on "Euthanasia." The first part is indicative of the range of the subject matters which are to be included.

The second part of volume one is dated October, 1955. There are seven articles and these also cover a wide field. Dr. R. Gwatkin writes on "Atrophic Rhinitis of Swine" and Dr. C. A. Hoare on "The epidemiological role of animal reservoirs in human leishmaniasis and trypanosomiasis." An account of "Nutrition and fertility in the bull" by Dr. D. E. Tribe and Mr. R. B. Cumming is followed by one on "Latent virus as exemplified by mouse hepatitis virus" by Dr. A. W. Gledhill and Dr. Janet S. F. Niven. "Muscular dystrophy" by Dr. K. L. Blaxter and Mr. R. F. McGill, "Vitamin 'A' and ovine rickets" by Dr. A. B. Grant and "Leptospirosis in farm animals" by Mr. N. R. McDonald completes this number.

The printing is good, the illustrations and figures are fairly clear and the parts are a convenient size. In most cases the articles have an authoritative stamp as they are written by individuals who have contributed original observations in the particular fields. Bibliographies are appended to the articles and so enhance their value.

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NEWS

"ATLAS" UNBREAKABLE NYLON SYRINGE NOW MADE IN 2 ml. SIZE

CHARLES F. THACKRAY, LTD., of Leeds, inform us that the "Atlas" range of unbreakable nylon syringes has been extended to include the popular 2 ml. size, the previous smallest in this range being 3 ml. Now, with the new addition, these syringes can be obtained in the following sizes: 2 ml., 3 ml., 5 ml., 10 ml., 20 ml.

The construction of the new 2 ml. syringe, though mainly similar to the larger capacities, has been adapted to meet general requirements. Thus, it incorporates a solid instead of a hollow plunger to prevent floating when boiled. For the same reason the 5 and 10 ml. sizes also are now made with heavy plungers, hollow plungers being available on request. In all other respects the new syringes have the same time-saving and cost-reducing features as the previous models. They are guaranteed unbreakable under normal conditions. Barrel and nozzle are made all in one. The piston is smooth working, leakproof and will not stick. The syringes can be sterilised by boiling or autoclaving at 18 lb. steam pressure (120° centigrade) and the pH of sera is not affected.

Prices of the new syringe are: 2 ml., 7s.; 3 ml., 9s. 6d.; 5 ml., 10s. 6d.; 10 ml., 12s. 6d.; 20 ml., 15s.

LOUPING ILL VACCINE CONCENTRATED

MESSRS. EVANS MEDICAL SUPPLIES LTD. advise us that the above product has now been added to their list and is available in rubber-capped bottles of 50 ml. (25 x 2 ml. concentrated doses). The price is as follows: Retail, 16s. 6d. each. (Subject to discount.)

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Letters for the Journal, literary contributions, reports, notices, books for review, exchanges, new instruments or materials, and all matter for publication (except advertisements) should be addressed to the Editor.

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